EFFECTS OF GIBBERELLIC ACID ON

MENTHA PIPERITA

by

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by

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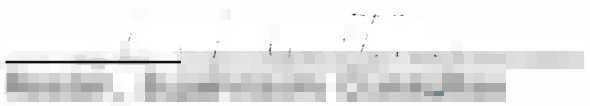

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INTRODUCTION

Rice is one of the world's staple foods and constitutes the basis of the diet for one half of the human race (Wissler, 1946). Any threat to the constant supply of rice is a threat to the well-being and life of millions of orientals. The "Bakanae" disease of rice is one of the most destructive to the crop, destroying in certain years over 40 per cent of the rice in Formosa (Sawada 1912). This disease is known by different names in various areas, "Foolish Seedling" being the translation most frequently given (Stodola, 1958).

According to Stowe and Yamaki (1957) rice plants affected with the bakanae disease often grow half again as tall as healthy seedlings. Such infected plants become more elongated and slender, and more pale than normal. In light cases of infection the rice plants sometimes grow to maturity and flower two or three days earlier than the normal plants, but the ears produced are small, and the yield of grain is much reduced. Often the plants with more severe infestations succumb to the disease with no production of fruit. Hori in 1898 identified an imperfect fungus as the causative agent. The Perfect stage of the bakanae organism was designated Gibberella fujikuroi (Sawada) Wollenweber and the imperfect stage as Fusarium moniliforme Winel. (Stodola, 1958). This plant belongs to the Division Eumycophyta (true Fungi), the class Ascomycetes (Sac Fungi) and to the family Tuberculariеae.

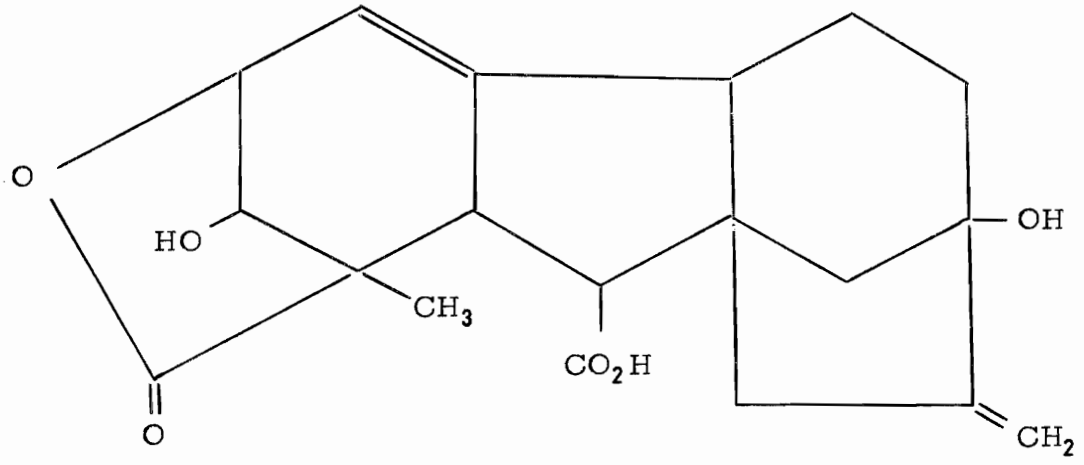
In 1926 Kurosawa, after several failures, succeeded in producing bakanae-like effects in rice seedlings by treating them with extracts from the culture media in which Gibberella fujikuroi (Saw.) Wr. had been growing (Kurosawa, 1926). Although Kurosawa's findings were published during the period of time when there existed considerable interest on the part of plant physiologists in plant growth regulators, interest in Kurosawa's findings was limited almost exclusively to Japan. The properties of the active principle were examined by several investigators as well as the optimum conditions for production of the material. A reliable rice seedling bioassay was developed at the University of Tokyo in 1935 (Stowe & Yamaki, 1957) and in 1938 Yabuta and Sumiki announced the isolation of two biologically active principles which they named Gibberellin A and B.

Mitchell and Angel (1951) were the first in the U.S.A. to investigate the gibberellins. Envisioning the potentialities of the gibberellins in

agriculture, Mitchell and Angel encouraged the study of the gibberellins by investigators trained in fermentation work (Stodola, 1958). Work commenced at the Northern Regional Laboratories of the U. S. Department of Agriculture. Apparently independently, a group at the Imperial Chemical Industries in Britain became interested in the gibberellins at the same time as the group from the U. S. Department of Agriculture, and much of the work of the Japanese was confirmed (Brian, 1959). Both the British and American work resulted in the isolation of gibberellins different from those reported by Yabuta and Sumiki. Subsequent work has resulted in the isolation and naming of a number of gibberellins.

Strains of Gibberella fujikuroi (Saw.) Wr. differ in their ability to produce the bakanae disease. They differ also in their capacity to produce gibberellins and in the proportions of the various gibberellins produced. The British chemists at Imperial Chemical Industries obtained only gibberellic acid from their fungal cultures (Borrow, et. al., 1955) while the chemists from the U.S. Department of Agriculture obtained a mixture of gibberellic acid and another gibberellin (Stodola, Nelson and Spence, 1957). After the development of suitable culture media and the isolation of the most active strains of fungus, Stodola (1956) concluded that commercial production would present no problem.

Gibberellin A of early Japanese chemists was a mixture of compounds, and was later separated into gibberellins A_1, A_2, and A_3, gibberellin A_3 being identical with gibberellic acid (Takahashi et. al., 1955). Stodola and co-workers (1955) isolated a compound in 1954 which they tentatively designated gibberellin X. When this compound was found to be identical with the gibberellic acid of Curtis and Cross (1954), the latter, more appropriate name was adopted. Gibberellic acid is the only gibberellin which has been thoroughly characterized (Stowe and Yamaki, 1957), and in 1956 Cross and co-workers proposed (1956) and later confirmed (1958) the following structural formula:



Plants do not respond in like manner to the various gibberellins, and so Stowe and Yamaki (1957) have suggested that the following abbreviations be used:[1]

For the gibberellins obtained from the original gibberellin A,

GA_1, GA_2, GA_3 (gibberellic acid)

An additional gibberellin has been isolated (Takahashi et. al., 1957) and has been designated GA_4.

Sufficient time has not elapsed for the suggestion to have been accepted or rejected; however, some articles appearing in the summer of 1959 do not include the proposed abbreviations. The most common abbreviation the author has noticed is GA and stands for either gibberellic acid or to a mixture of gibberellins. Probably most of the commercial material designated as gibberellic acid or as GA is in reality a mixture of gibberellins (Brian, 1959).

Almost no physiological responses have been noted in animals treated with gibberellins (Peck et. al., 1957, Eli Lilly & Co. 1957, Kimura et. al., 1959). A number of physiological responses have been obtained in plants by the application of gibberellins. Stem elongation is the most typical, and most biological assays for gibberellins are based upon this property (Brian and Hemming 1955, Eli Lilly & Co., 1957). Wittwer and Bukovac (1958) listed "Twenty-five physiological responses in selected higher plants controlled by gibberellin heretofore not subject to chemical regulation." Stodola (1958) include the effects of gibberellins on plants under the following headings:

1. Increased vegetative growth
2. Reversal of dwarf habit
3. Induction of flowering
4. Effect on fruiting
5. Effect on seed germination
6. Effect on dormancy of vegetative organs
7. Other effects

"Gibberellin Fact Book" by Eli Lilly and Co., (1957) lists the following under "general response of plants to gibberellin":

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[1]Gibberellin B, as reported by Yabuta and Sumiki (1938), must be a synonym with one of the above gibberellins or mixtures, as it is not recognized as a separate gibberellin (Brian, 1959).

Gibberellin makes plants grow taller.
Gibberellin increases the total weight.
Gibberellin breaks dormancy.
Gibberellin stimulates germination.
Gibberellin increases fruit set.
Gibberellin produces some undesirable effects.

At the present there seems to be no generally-used system of classifying the large number of responses of plants to gibberellins.

While the positive results are most interesting and reports of them appear in the literature, it should be noted that not all plants are affected by treatment with gibberellins. For example, while certain dwarf strains of maize treated with gibberellins grow approximately as large as normal, other dwarf strains of the same species do not respond at all (Phinney, 1956). Certain horticultural varieties of Japanese chrysanthamum (Chrysanthemum morifolium) bloom earlier following treatment, but the time of blossoming in other varieties is not altered (Harada and Nitsch, 1959). Only after the application of gibberellins is it possible to state whether or not a given effect will be elicited in a particular plant.

Though many responses have been observed in plants, authors have been willing to offer few recommendations for the use of gibberellins in crop production up to the present time. Wittwer and Bukovac (1958) state regarding gibberellin, "no one is able to predict its ultimate value at this time."

The caution "Not to be construed as recommendations" is included under the heading of a comprehensive table summarizing "Promising uses for gibberellin in crop production." Publications by manufacturers of gibberellic acid include the following: "Larger scale experiments are necessary before a true assessment of its practical value can be made. This section of the Technical Data Sheet is intended to focus attention on those crops on which extended trails seem to be most worthwhile" (Plant Protection Ltd., 1957). In another publication (Eli Lilly & Co., 1957), the recommendations listed are for three species for production of ornamental flowers, one specie for production of forage crops, and six species of vegetables for seed production.

Despite the conservative attitude toward the recommendations for the use of gibberellins outside of experimental work, many uses are anticipated. Wittwer and Bukovac (1958) have stated "The favorable modifications of plant behavior resulting from treatment with gibberellin may well exceed the anticipation of those who have been most active in developing its potential agricultural uses." Because of the low order of toxicity of gibberellin to mammals, the Federal Food and Drug Administration has released gibberellic acid for use on plants intended for food and animal forage.

Following application with gibberellins, in the plants that respond to treatment with an increase in stem length, there is sometimes an increase in dry weight over the untreated plants (Leben and Barton, 1957, Bukovac and Wittwer, 1956, Morgan and Mees, 1956, Marth et al, 1956, Sayed and Beal, 1959). The first paper reporting changes in plant constituents was that of Hayashi (1940) in which it was noted that gibberellins increased the amount of amylase in germinated grainsof barley and wheat. Following this, changes in the amount of various plant constituents have been reported.

Working with gibberellin-treated rice seedlings, Yabuta, et. al. (1951) found total carbohydrates reduced; and Hayashi, et. al., (1953) reported that sucrose and starch were decreased but that hemicellulose and cellulose were somewhat increased. Following treatment of plants with gibberellins, Brian (1954) found an increase in soluble carbohydrates, particularly of glucose, in peas and wheat. Imperial Chemical Industries, Ltd., (1955) reported increases in fructose and glucose in wheat seedlings; and Wittwer, et. al., (1957) indicated that total sugars were significantly decreased in Kentucky Bluegrass.

Stutz and Watanabe (1957), working with Lupines, and Pilet (1957), working with carrot tissue, reported lowered IAA-oxidace activity. Weller, et. al., (1957) observed lowered phosphatase activity in young bean plants, and also changes in β -amylase activity which were different for various parts of the plant.

A few reports have considered the changes in the amount of active drug constituents which are normally obtained from plants. Yabuta and co-workers (1943) found that the nicotine content of tobacco leaves was reduced following gibberellin treatment. Smith and Sciuchetti (1959) found the total plant alkaloids of Stramonium was increased by 46 per cent, but that the total plant alkaloids in belladonna was decreased by about one-half by treatment with gibberellic acid. Masuda and Hamor (1959) found an increased yield of total plant alkaloids in hyoscyamus following treatment with gibberellins. Sayed and Beal (1959, 1959a) obtained an increase in rutin content per plant and an increase in per cent of glycosides in Fagopyrum esculentum and Digitalis purporea, respectively.

The author has seen no literature to date on the effects of gibberellins on many classes of plant constituents such as volatile oils, fixed oils, balsams, resins, and gums. Because of the economic importance of volatile oil-bearing plants in the field of pharmacy, it was decided to investigate the effects of gibberellic acid on peppermint (Mentha piperita L.). It was decided that the following questions would constitute the primary consideration: (1) Will the application of gibberellic acid effect the production of peppermint herb and peppermint oil, and (2) providing an effect is observed, will the resulting products meet U.S.P. specifications.

GENERAL PROCEDURES

A. SOURCE OF PLANT MATERIAL

The peppermint plants (Mentha piperita L.) used in this investigation were divisions grown from a single plant obtained from the drug plant garden of the University of Colorado College of Pharmacy. The original plant was divided into clones during the summer of 1957, and during the winter of 1957-58 these clones were further divided and grown in pots and benches in the greenhouses at the University of Utah. In the spring of 1958, about 100 clones were taken from the greenhouses and planted in a garden in Centerville, Utah. In the fall of the same year, some of the plants were brought back into the greenhouses. In the spring of 1959, some of the clones that had been left in the garden through the winter were further divided, giving a total of about 150 clones. Observations and measurements were made on the plants in the greenhouses during the winters of 1957-58 and 1958-59. During the summers of 1958 and 1959, collections were made of field-grown peppermint herb and the volatile oil extracted.

The peppermint plants in the greenhouses were grown in a rich loam soil and the plants in the garden were grown in a sandy-gravelly loam. The garden is situated at the base of the Wasatch range, about 100 feet above the valley floor. The garden slopes to the West, and diggings in the area indicate that there is at least 60 feet of sand and gravel underlying the topsoil. Irrigation was necessary throughout the frost-free days of the year.

Irrigation water, obtained from a small mountain creek every fourth day, was applied to the garden by flooding. This amount of irrigation was inadequate to keep the peppermint plants from wilting, and so supplimentary sprinkling from the Centerville city water supply was found necessary. Despite efforts to keep the soil around the peppermint plants moist, the plants treated with gibberellic acid wilted on a number of days. As soon as wilting was noted, all peppermint plants were sprinkled with water. The plants regained their normal appearance within a short time following the additional water. The plants treated with the highest concentration of gibberellic acid wilted more readily than other plants. Wilting of the control plants was never noted. Probable explanations for this wilting will be given in the discussion.

B. SOURCE OF GIBBERELLIC ACID

The gibberellin used throughout the experiments was obtained from Merck & Company, as GIBREL, the "potassium salt of gibberellic acid." According to Brian (1959), the gibberellin products used in the U.S.A. is a mixture of gibberellins, being about half gibberellic acid and half gibberellin A_1. This mixture is referred to as gibberellic acid in the literature and the abbreviation GA is frequently used. The abbreviation GA will be used throughout the paper.

C. APPLICATION OF GIBBERELLIC ACID

Aqueous spray of 0, 10, and 100 ppm. GA were used in the experiments. Portions of GA were accurately weighed and dissolved in sufficient absolute alcohol so that one mililiter of the resulting stock solution when diluted to 100 ml. with water produced the appropriate concentration. The aqueous solutions were prepared from the alcoholic stock solutions immediately before use and in all cases were applied to the plants within an hour of the time they were mixed. The following advantages were realized by preparing an alcoholic stock solution: (1) There was no reduction in potency due to deterioration of the GA (Plant Protection Ltd., 1957). (2) The aqueous solutions were rapidly and simply prepared immediately prior to the time they were used. (3) Small quantities of solution were prepared with much less error due to chance than could have been prepared if the GA had been weighed out for each solution.

The solutions were sprayed on the peppermint plants until the solutions began to drip from the leaves. The size of the plant, therefore, determined the quantity of solution used. On small plants, as little as one or two mililiters (representing 10 or 20 mcg. of GA in the 10 ppm solution) was required to produce runoff from the leaves but larger plants required up to 30 or 40 times as much.

The following precautions were taken to minimize the possibility of spray drifting from one plant to another:

(1) Plants were planted about two feet apart.
(2) A very coarse spray was used.
(3) A three-sided cardboard shield with each side about one meter square was held between the plant being sprayed and the plants next to it.
(4) Spraying was done only when the air was fairly calm, generally in the early morning. Whenever there was enough air movement that drifting of the spray might have occurred, the spraying was postponed until the following evening or morning.

In previous experiments it had been noted by the author that the effects (at least in peppermint) were manifest only on the stems that were sprayed with GA, and that the GA apparently did not travel from one stem through the rhizomes and up into other stems.[1] Every effort was made to see that the leaves of all stems were treated equally.

D. COLLECTION OF VOLATILE OILS

When the peppermint plants first started to bloom they were harvested. In order to insure maximum yields, the plants were cut at ground level by the use of a hand pruning shears. The whole fresh tops were used in the extraction process. In order to obtain samples of volatile oil from reasonably large amounts of peppermint, a modified Lloyd extractor was used (Cole 1956). The plant material was placed in a cotton cloth sack so that it could be more easily placed in and removed from the retort. The sack of plant material in the retort was covered with water and the retort cover was closed. Steam at about two pounds of pressure was introduced into the jacket surrounding the retort, producing rapid boiling of the water around the peppermint. As the volatile oil and steam condensed, the oil floated up into a gauge glass below the condensor and excess water was returned to the retort by a cohobation device. It was noted that most volatile oil was obtained in the first half hour of condensation, and that after five hours of boiling, no odor of peppermint oil could be detected in the mark. In all cases the distillation was continued for five hours.

Following distillation, the material from the oil trap was drawn off into a separatory funnel and most of the water removed. Because small quantities of oil remained in the oil trap, three, ten mililiter portions of ether were run through the Lloyd extractor in order to flush out the remaining oil. The ether was evaporated from the mixture under partial vacuum.

The material which collected in the oil trap of the Lloyd extractor was greenish-yellow in color and contained substances that upon redistillation were nonvolatile. Upon redistillation in a clevenger apparatus for the assay of oils lighter than water, a colorless volatile oil portion was obtained as well as a solid, nonvolatile, resin-like substance. The resin-like substance was discarded and the volatile oil was washed out of the oil trap and into a separatory funnel with ether. From the separatory funnel, the ether-volatile oil portion was placed in a flask and the ether evaporated off under partial vacuum. The oil was dried with anhydrous salts and filtered.

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[1] This is not entirely in agreement with the report of Neely and Phinney (1957) and Persson and Rappaport (1958), who indicated the GA travels through lateral stems as well as up and down the axis of the stem treated with GA. Different plants were used by these authors, however, and underground rhizomes were not involved.

Some of the plant material was assayed for oil content by the National Formulary IX method (1950). In most cases, about 300 Gm. of plant material was placed in a 12-liter, round-bottomed flask along with sufficient water to cover the plant material. The flask was placed in an electric heating mantle and the mixture was boiled until no odor of volatile oil could be detected. In all cases, boiling was discontinued after five hours. The oil was collected in a volatile oil trap designed for oils lighter than water (Clevenger 1928). The trap was filled with water previous to the distillation in order to prevent droplets of oil from collecting in the water along the sides of the tubes. The amount of oil was read directly from the calibrated tube. Less oil was obtained from the GA-treated plants. Further details are included in the investigation.

E. MICROSCOPIC PROCEDURES

Plants used in the microscopic examination of the powdered crude drugs were grown during the summer of 1958, and were randomly selected from the freshly-cut material used in obtaining the volatile oil for assay purposes. They were collected July 7 and 8, and air dried in the pharmacognosy laboratory at the University of Utah. After two weeks of drying, they were placed in closed, dry jars and kept until February, 1959, when they were powdered using a micro-Wiley mill and a 40-mesh sieve. The powdered material was then examined, using water, chloral hydrate T.S., and phloroglucinol T.S. as suspending agents.

Microscopic sections of peppermint were made from plants grown in the greenhouses during the winter of 1958-59, and from plants grown in the garden during the summer of 1959. The plants from which the sections were made were carefully matched to make sure that, insofar as possible, the leaves and stem sections were an equal number of nodes from the ground and from the growing tip; that they were the same age, at the same stage of development, were from the side of the clump receiving equal amounts of light and moisture, and were in every way as analagous as possible, except for the treatment given.

Leaf and stem material were killed and fixed in Formalin-Aceto-50% alcohol mixture and de-aerated by means of a faucet aspirator. Dehydration was in a series of ethyl alcohol concentrations and the sections were then prepared for imbedding in a rubber-paraffin mixture. Twenty micron-thick stem cross sections, stem longitudinal sections, and leaf cross sections were made using a rotary microtome. The sections were secured to glass slides by the use of Haupt's adhesive and were later stained by the use of Safranin-fast green method (Flowers, Johansen 1940). Cover slips were mounted using a synthetic resin, "Permount."

INVESTIGATION I. EFFECT OF GIBBERELLIC ACID ON MORPHOLOGY OF PLANTS

A. EFFECT ON STEM ELONGATION

Increased stem elongation is noted universally in plants that respond to GA (Brian 1959), and therefore stem elongation may be taken as an indication as to whether or not GA will have an effect upon a given plant. It was noted that the stems of GA-treated peppermint plants elongated more rapidly than the controls (see Plate 1).

As soon as vigorous growth was established in the peppermint plants that had been transplanted from the greenhouses to the garden in the spring of 1958, each clump of peppermint was randomly assigned to receive either 0, 10, or 100 ppm of GA. The plants sprayed with an aqueous solution containing no GA will be referred to as the control. The plants were marked with stakes and sprayed weekly with the appropriate concentration of GA. After eight weeks of spraying, and just previous to the harvest of the peppermint, the following measurements were taken:

(1) Length of tallest stem in each clump of peppermint.
(2) Average height (estimated by sight) of all stems of each clump of peppermint.
(3) Average internode length on twenty randomly-selected stems from each treatment.

Figures 1 and 2 show graphically the differences in the measurements taken of plants treated with 0, 10, and 100 ppm of GA. An analysis of variance for each of the measurements indicates that the increased growth induced by GA spraying is highly significant.

At time of treatment.

One week following treatment.

One month following treatment.

PLATE I Growth response of peppermint to gibberellic acid. The plants from left to right received 100, 10, and 0 ppm GA.

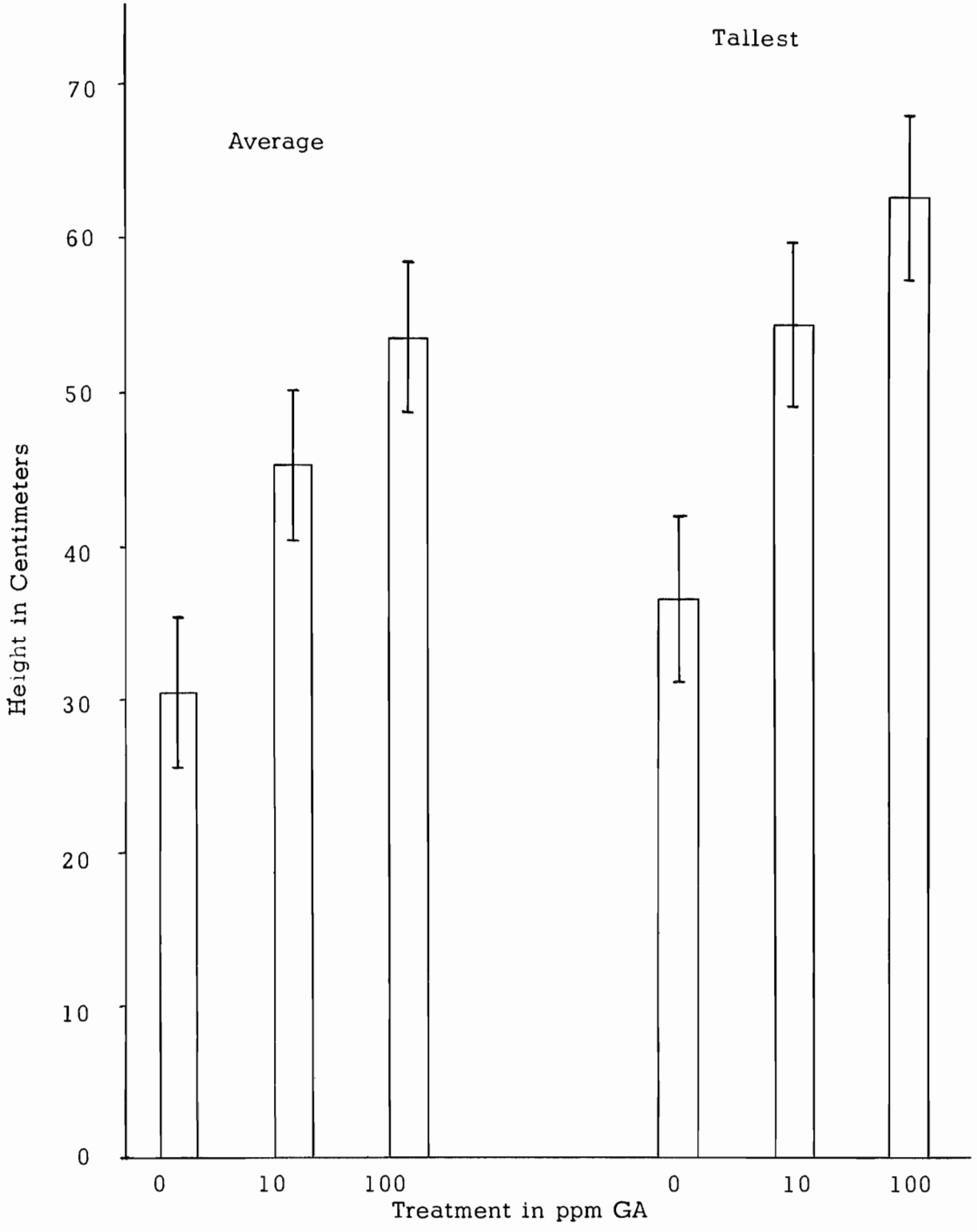


Fig. 1 Height of tallest stem and average height of stems from each clump of peppermint at time of harvest. The 95 per cent fiducial limits are indicated by the vertical bracketed bars. See text for discussion.

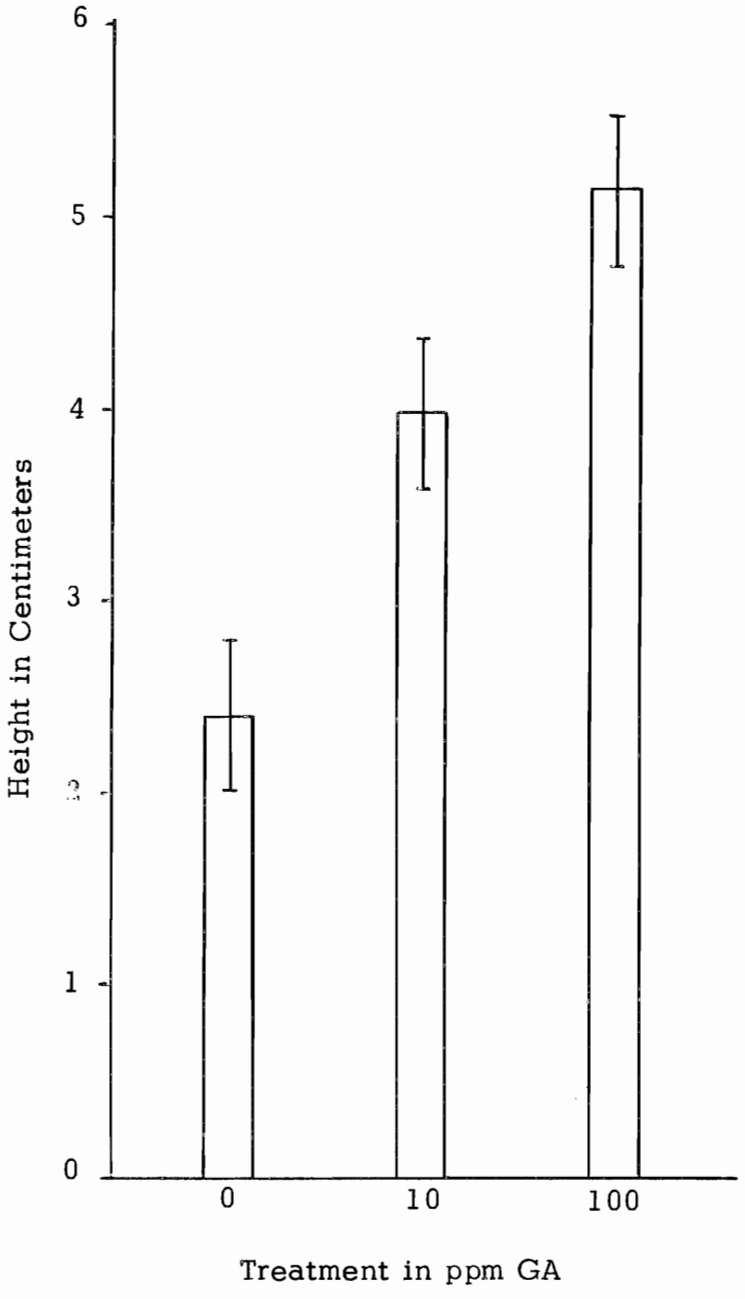


Fig. 2 Average internode length of randomly selected peppermint stems at time of harvest. The 95 per cent fiducial limits are indicated by the vertical bracketed bars. See text for discussion.

Table 1

Analysis of Variance for Height of Tallest Stem

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	3,618	
Between Samples	2	2,795	1,398**
Error	21	823	39.17

** Significant at 0.01 level

Calculated F = 35.7

Theoretical $F\begin{bmatrix}2\\21 & 0.01\end{bmatrix} = 5.9$

Table 2

Analysis of Variance for Height of Average Stem

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	23	2,826	
Between Samples	2	2,214	1,107**
Error	21	612	29.14

** Significant at 0.01 level

Calculated F = 38.0

Theoretical $F\begin{bmatrix}2\\21 & 0.01\end{bmatrix} = 5.9$

Table 3

Analysis of Variance, Length of Average Internode

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	59	90.94	
Between Samples	2	68.53	34.26**
Error	57	22.41	0.3932

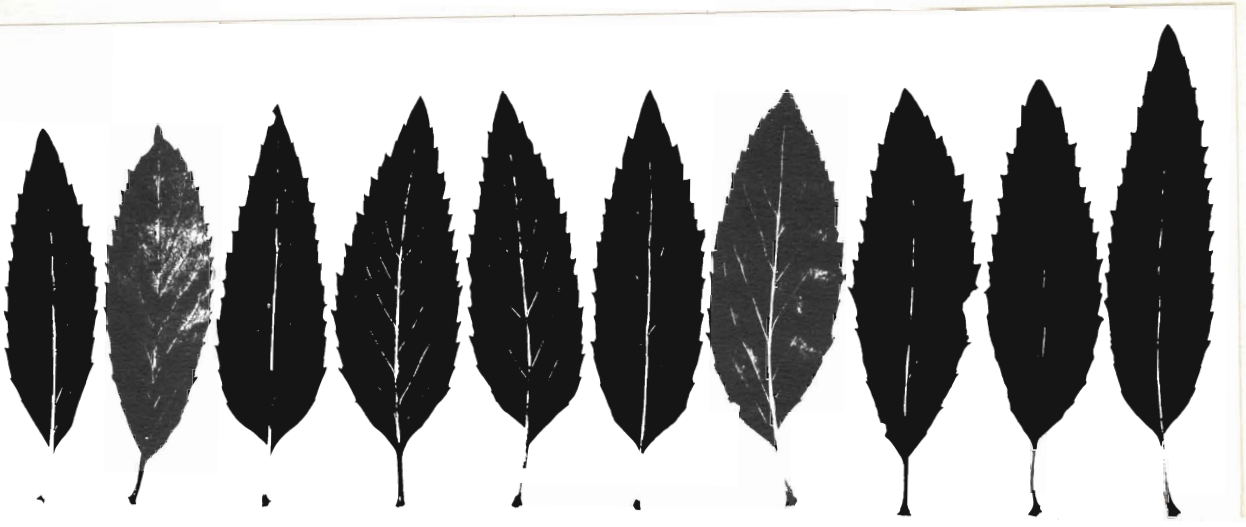
** Significant at 0.01 level

Calculated F = 87.2

Theoretical $F\left[\begin{matrix}2\\57\end{matrix}\ 0.01\right] = 5.4$

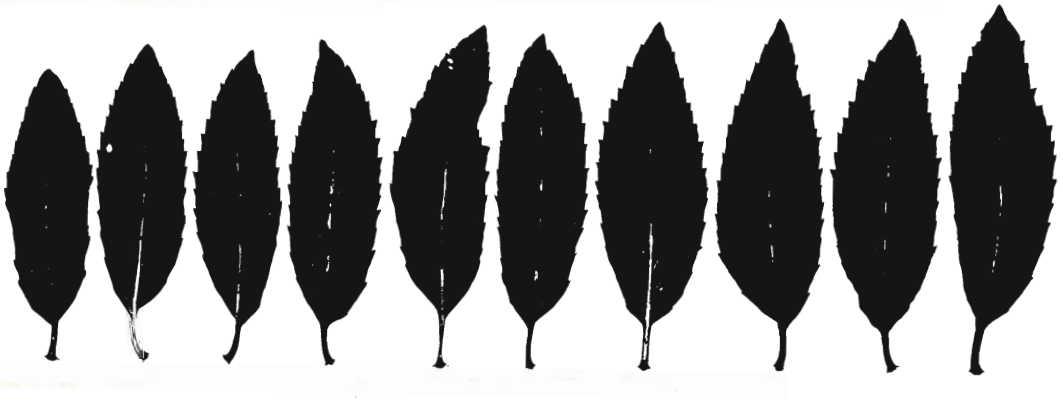
B. EFFECT ON LEAF SHAPE

At the same time that measurements for stem elongation were made, the leaf shape was noted and 100 leaves from each treatment were pressed so that the leaf area could be calculated. The shape of leaves from the GA-treated plant differed from the control plants according to the position of the leaf on the stem. Plate II shows the shape of the leaves taken from the fourth or fifth node above the ground. They are similar in shape to the leaves of the control plant except that they are slightly larger and the petioles are slightly elongated. Plate III shows the shape of the leaves two or three nodes below the flower cluster. They are smaller in area and more lanceolate in outline. An analysis of variance indicates that the "lower" leaves are significantly larger in area than the control leaves but that the "higher" leaves are significantly smaller in area than the control leaves.



From plants treated with 100 ppm GA.

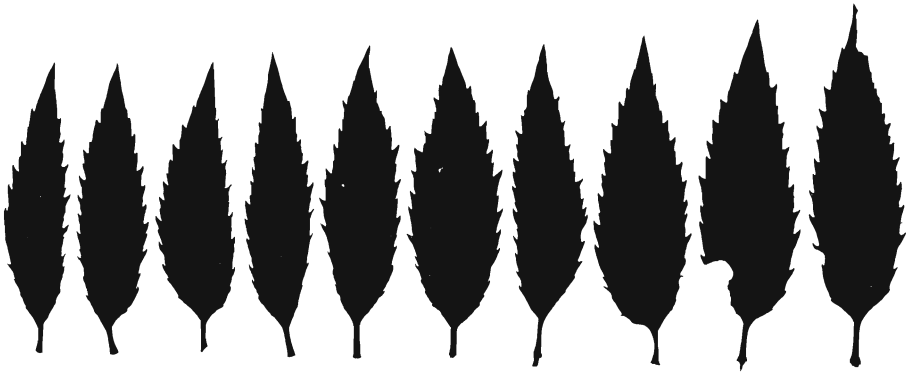
From plants treated with 10 ppm GA.



From plants treated with 0 ppm GA.

PLATE II "Lower" leaves from plants treated with 100, 10, and 0 ppm GA. See text for discussion.

From plants treated with 100 ppm GA.



From plants treated with 10 ppm GA.

PLATE III · "Higher" leaves from plants treated with 100, 10, and 0 ppm GA. See text for discussion.

Table 4

Analysis of Variance for Size of "Higher" Leaves

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	15,194	
Between Samples	2	7,192	3,596**
Error	27	8,002	296

** Significant at 0.01 level

Calculated F = 12.1

Expected $F\begin{bmatrix}2\\27\end{bmatrix}_{0.01} = 5.5$

Table 5

Analysis of Variance for Size of "Lower" Leaves

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	134,000	
Between Samples	2	88,620	44,309**
Error	27	45,410	1,682

** Significant at 0.01 level

Calculated F = 26.3

Expected $F\begin{bmatrix}2\\27\end{bmatrix}_{0.01} = 5.5$

C. EFFECT ON LEAF NUMBER

At the same time that measurements for stem elongation were made, counts were made of the number of leaves per stem on 50 stems from each treatment. Table 6 gives the average number of leaves on the stems from plants subjected to each treatment. An analysis of variance indicates that the differences are not significant.

Table 6

Average Number of Leaves Per Stem

Treatment	Number Leaves
0 ppm	33.8
10 ppm	36.56
100 ppm	36.8

Table 7

Analysis of Variance for Number of Leaves

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	149	2,470	
Between Samples	2	71	35.84
Error	147	2,399	16.32

Calculated $F = 2.2$

Expected $F \begin{bmatrix} 2 \\ 147 \end{bmatrix} 0.05 = 3.1$

D. EFFECT ON WEIGHT

During the spring of 1959, thirty peppermint plants of similar size were randomly assigned to ten groups of three plants each. One plant of each group was sprayed weekly with either 0, 10, or 100 ppm of GA. On the 24th of June, as the first flowers were starting to appear, each plant was harvested and the fresh weight recorded. Per cent volatile oil was determined for each plant following the N.F. IX procedure (National Formulary 1950). Following the extraction of volatile oil, the plant material was quantitatively removed from the distillation flask and the entire contents dried to constant weight at 100° - 105° C. in a drying oven. As this dried material included not only the water insoluble but also the water soluble material, it was an accurate measure of the total non-volatile dry weight.

Figure 3 shows the fresh weights and also the dry weights of the plants resulting from the three treatments. An analysis of variance indicates that the differences in wet weights are significant at the 0.05 level, but that the differences in dry weight are not significantly different ($P < 0.05$).

Table 8

Analysis of Variance for Fresh Weight Per Plant

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	81,400	
Between Samples	2	19,820	9,907 *
Error	27	61,580	2,281

* Significant at 0.05 level

Calculated F = 4.3

Expected $F\left[\begin{matrix}2\\27\end{matrix}\ 0.05\right] = 3.4$

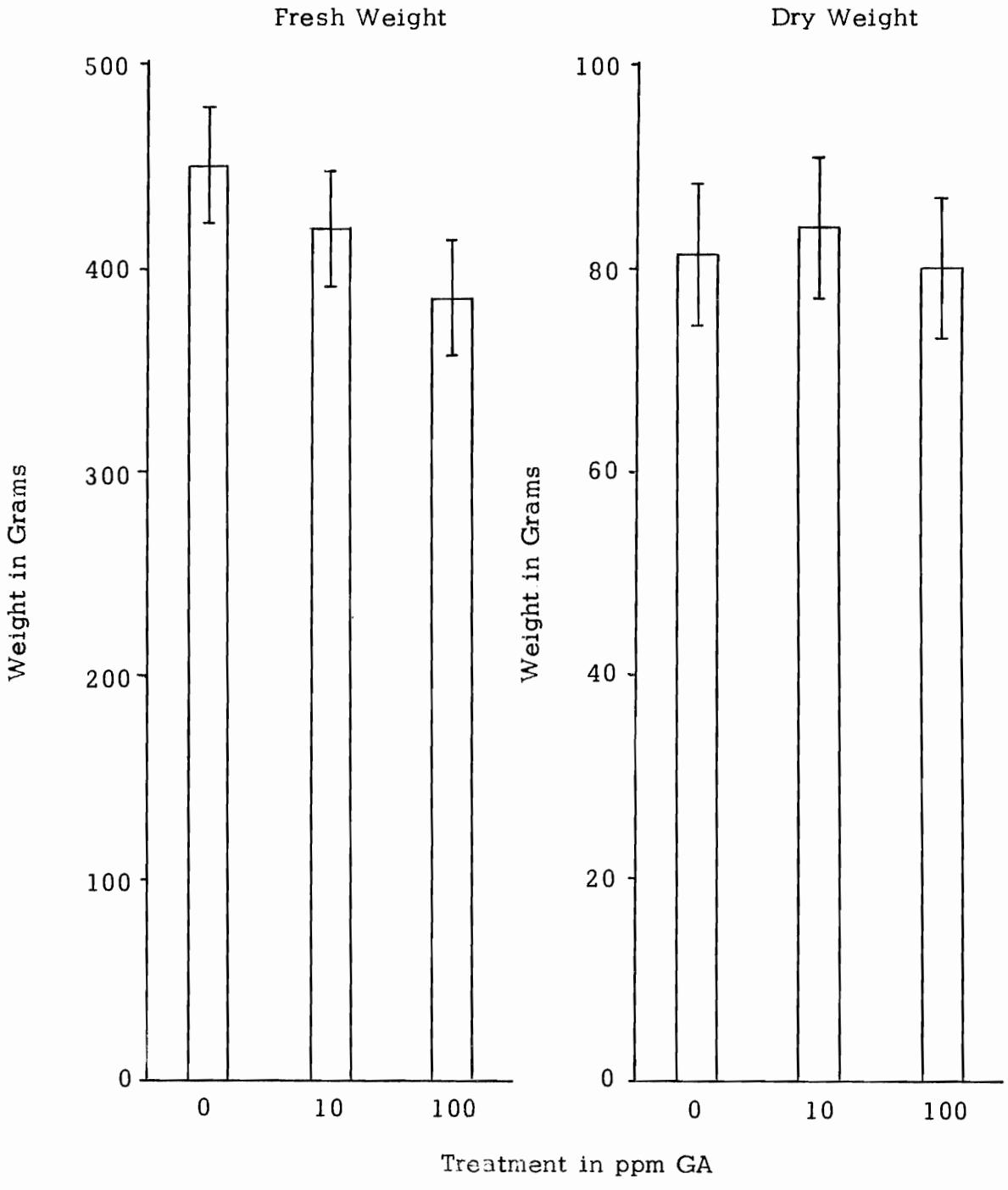


Fig. 3 Fresh weight and dry weight yield of peppermint herb per plan
The 95 per cent fiducial limits are indicated by the vertical
bracketed bars. See text for discussion.

Table 9

Analysis of Variance for Dry Weight Per Plant

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	3,639	
Between Samples	2	73	36.64
Error	27	3,566	132.10

Calculated F = Less than one

Expected $F\begin{bmatrix}2\\27\end{bmatrix}_{0.05}$ = 3.4

E. EFFECT ON VOLATILE OIL CONTENT

The volatile oil yield was compared on the basis of volatile oil per plant, per 100 Gm. of fresh weight, and per 100 Gm. of dry weight of peppermint herb. Figures 4 and 5 illustrate the reduction in oil yield. An analysis of variance indicates that the reduction in oil yield is significant ($P > 0.01$) in all cases.

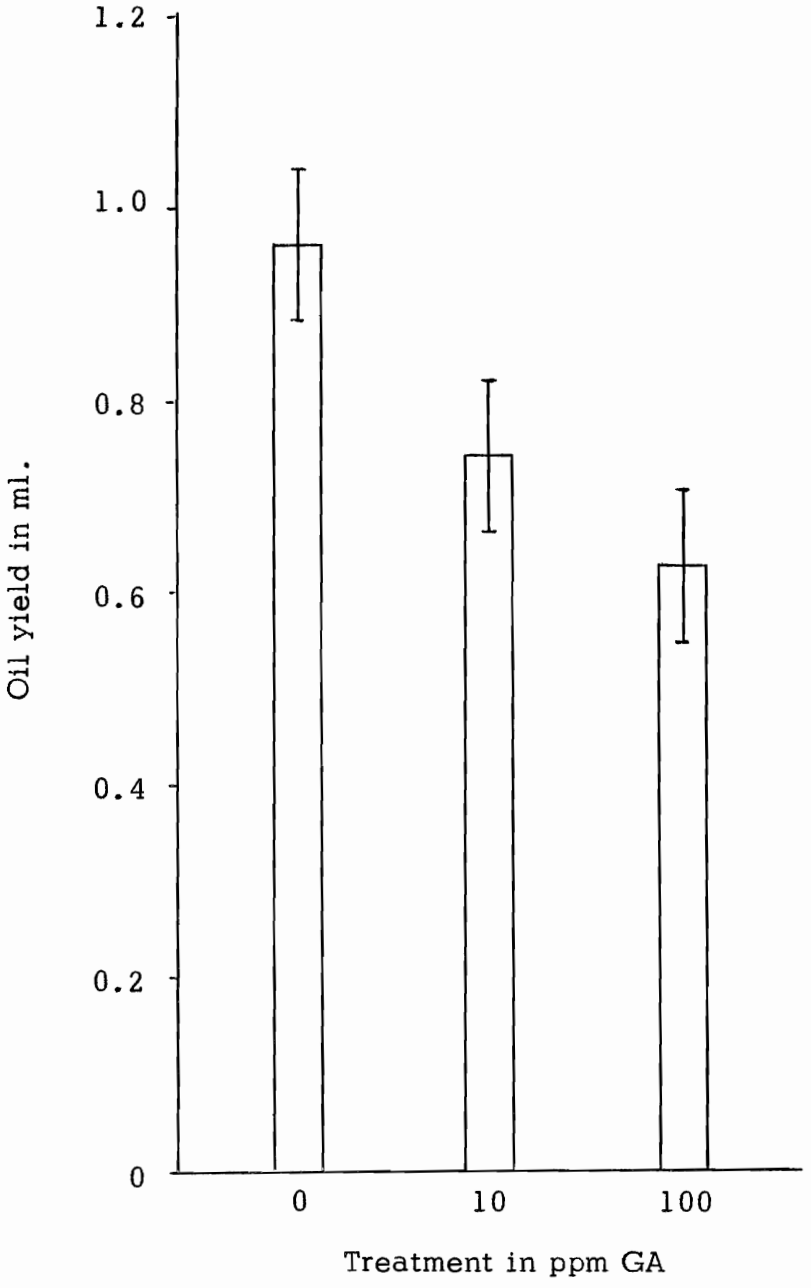


Fig. 4 Volatile oil yield per peppermint plant. The 95 per cent fiducial limits are indicated by the vertical bracketed bars. See text for discussion.

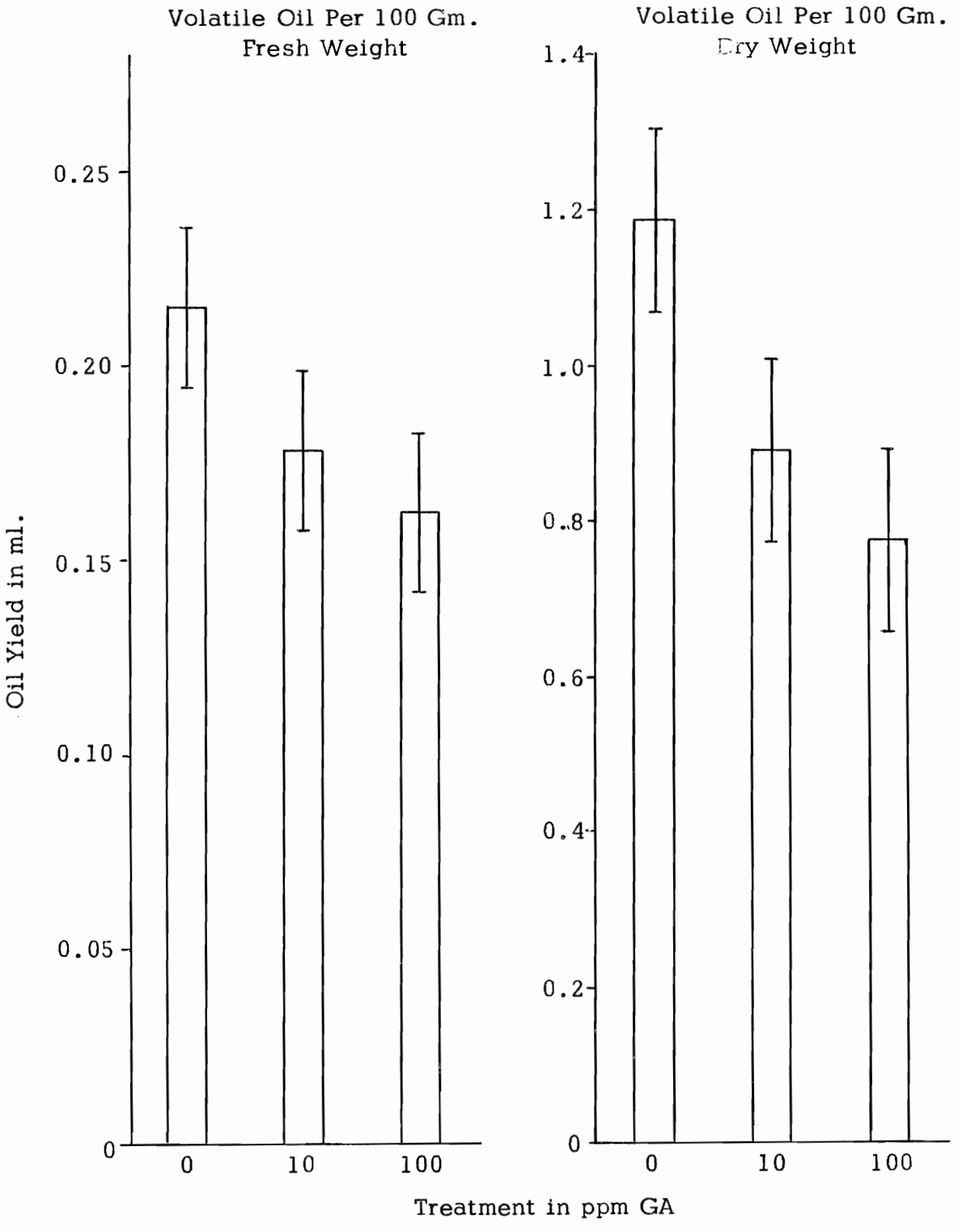


Fig. 5 Volatile oil yield per 100 Gm. fresh weight and volatile oil yield per 100 Gm. dry weight of peppermint herb. The 95 per cent fiducial limits are indicated by the vertical bracketed bars. See text for discussion.

Table 10

Analysis of Variance for Volatile Oil Per Plant

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	1,002	
Between Samples	2	0.572	0.286,0**
Error	27	0.430	0.021,5

** Significant at 0.01 level

Calculated F = 13.3

Expected $F\begin{bmatrix}2\\27 & 0.01\end{bmatrix}$ = 5.5

Table 11

Analysis of Variance for Volatile Oil Per 100 Gm.
Fresh Peppermint Herb

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	0.022,46	
Between Samples	2	0.014,12	0.007,061**
Error	27	0.008,34	0.000,309

** Significant at 0.01 level

Calculated F = 22.9

Expected $F\begin{bmatrix}2\\27 & 0.01\end{bmatrix}$ = 5.5

Table 12

Analysis of Variance for Volatile Oil Per 100 Gm.
of Dry Peppermint Herb

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	1.209	
Between Samples	2	0.873	0.4369**
Error	27	0.335	0.0124

** Significant at 0.01 level

Calculated F = 35.1

Expected $F\left[\begin{smallmatrix}2\\27\end{smallmatrix}\ 0.01\right] = 5.5$

F. EFFECT ON RECOVERY FOLLOWING HARVEST

During the winter of 1958-59 the observation was made that, following harvest of GA-treated plants, there appeared to be a reduction in vigor and growth of peppermint (See plate IV). In order to analyze this reduction in growth statistically, the 30 plants harvested in the summer of 1959 were allowed to recover for three weeks. The plants were not sprayed with GA during this three-week period and were then harvested. The material was weighed at the time of harvest. Figure 6 shows the reduction in peppermint herb production in the GA-treated plants. The reduction was significant ($P > 0.01$).

At time of first harvest, one month following treatment.

One month following first harvest, two months following treatment.

PLATE IV Growth response of peppermint to gibberellic acid. The plants from left to right received 100, 10, and 0 ppm GA. See text for discussion.

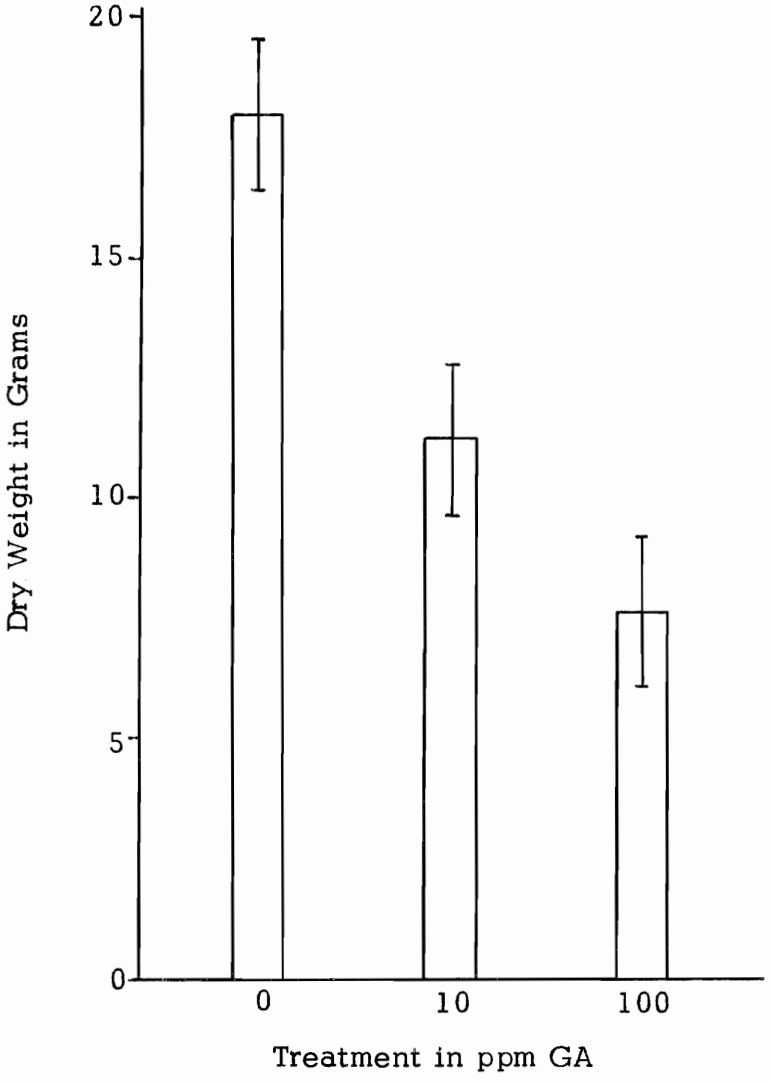


Fig. 6 Dry weight yield of peppermint herb three weeks following harvest of GA-treated and control plants. The 95 per cent fiducial limits are indicated by the vertical bracketed bars. See text for discussion.

Table 13

Analysis of Variance for Recovery Following Harvest

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square
Total	29	6.770	
Between Samples	2	5.182	2.5911**
Error	27	1.588	0.0588

** Significant at 0.01 level

Computed F = 44.1

Expected $F\begin{bmatrix}2\\27\end{bmatrix}_{0.01} = 5.5$

G. EFFECT OF A SINGLE SPRAYING OF GIBBERELLIC ACID ON STEM ELONGATION

During the winter of 1958-59, four groups of three potted peppermint plants each were chosen so that within each group, the plants were of similar vigor, had an equal number of shoots per plant, and the stems were of equal height. Each of the three plants in a group were randomly chosen to receive a single spraying of either 0, 10, or 100 ppm of GA. Each week measurements were taken of the height of vigorously growing stems in each pot. A total of 51 stems were measured, 17 for each of the three treatments. After eight weeks had elapsed and at the time flowers were opening, the stems were cut and measurements made on the internode lengths.

Figure 7 shows the average heights of the plants each week over the 8-week period, and Figure 8 shows the internode lengths of an average stem for each of the treatments at the time of harvest. It may be noted that stems of plants treated with GA elongated more rapidly for three or four weeks and that following this the rate of stem elongation was retarded for a short time. The length of internodes being produced was greater than normal immediately following treatment; however, internodes produced later were shorter than normal. A probable explanation is included in the discussion.

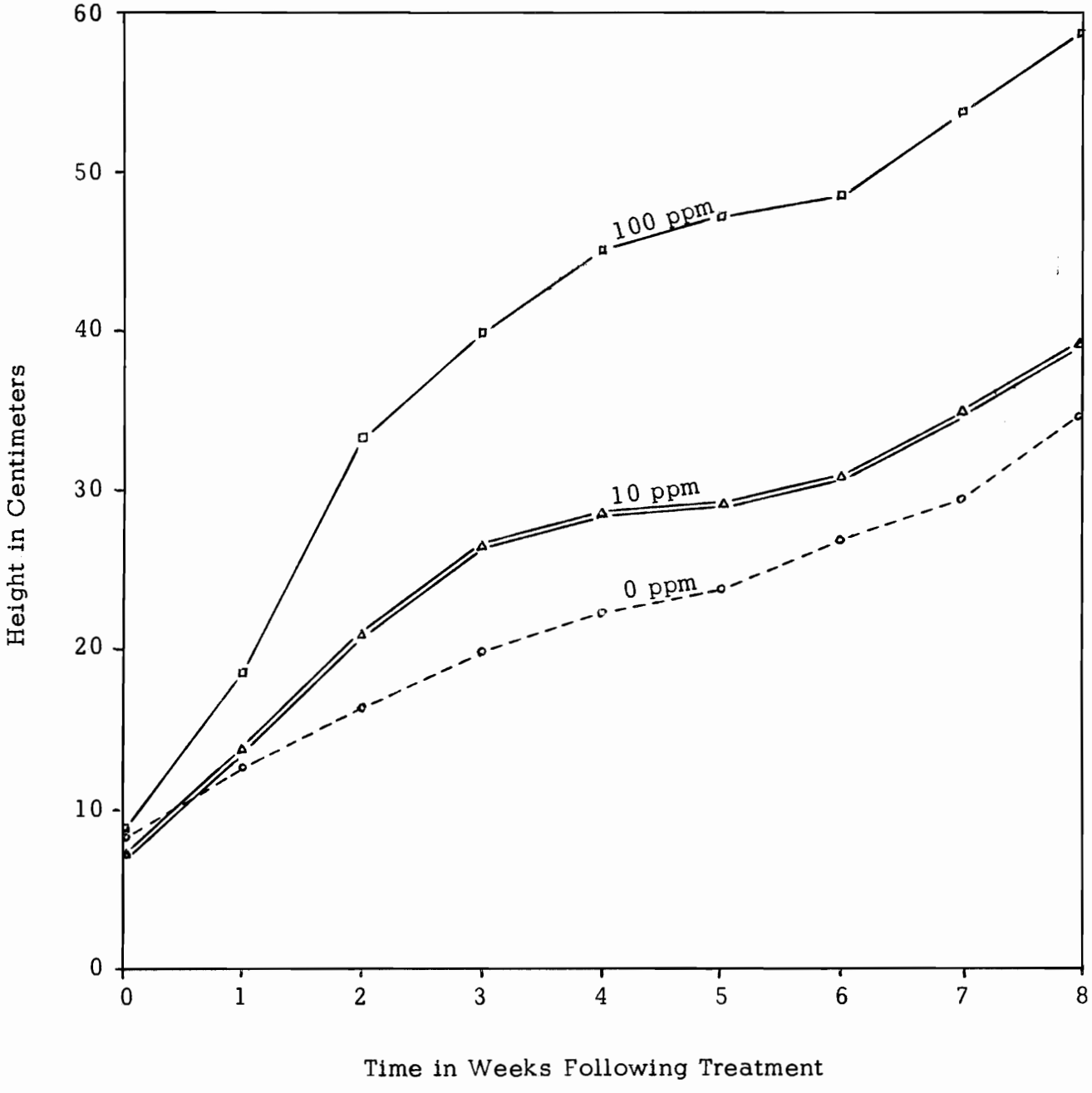


Fig. 7 Effect of one treatment on the height of peppermint plants during eight weeks. See text for discussion.

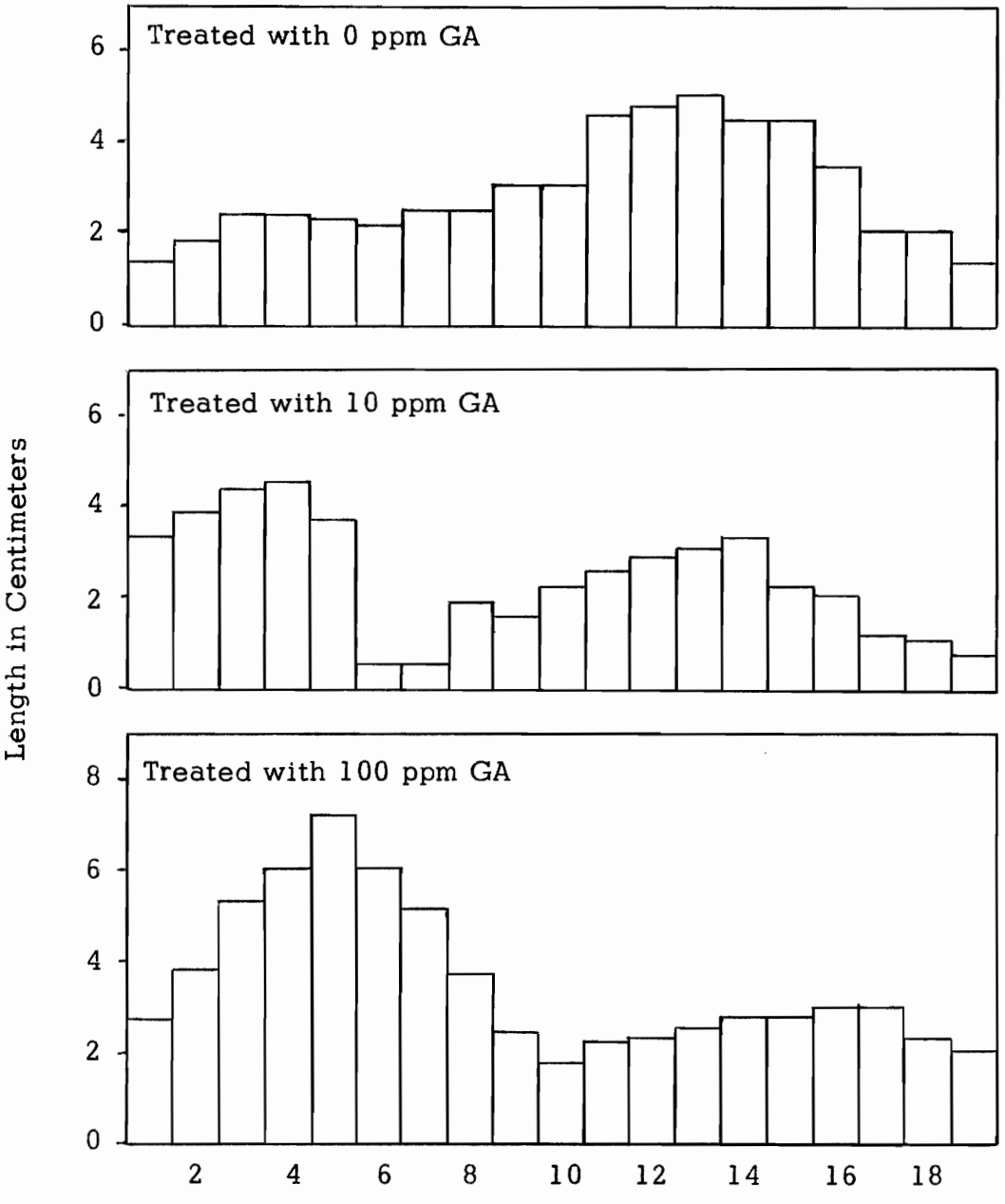


Fig. 8 Successive internode lengths along a typical stem following a single treatment. See text for discussion.

H. OTHER EFFECTS ON FIELD-GROWN PLANTS

In addition to the changes already mentioned, the following observations were made regarding the treated peppermint plants as compared with the controls:

(1) They were slightly chlorotic in appearance. This chlorotic appearance was most obvious early in the spring and was less conspicuous following applications of nitrogen-rich fertilizers to the garden.

(2) The leaves stood more erect, that is, the leaves formed a smaller angle with the stem. This was most noticeable at the top 10 to 15 centimeters of the plants, and was less obvious lower down.

(3) Flowering occurred at approximately the same time as the non-treated plants, and the flowers appeared equal in number and shape.

(4) There were fewer rhizomes produced.

(5) There was more winter killing.

(6) The stems were thinner, and the plants appeared more spindly.

(7) There was more evidence of insect feeding on the leaves of the plants.

(8) There was more wilting noted on hot, dry, windy days. As the plants approached blooming size, wilting could be prevented in the plants sprayed with 100 ppm GA only by daily irrigation of the garden.[1]

(9) The stems grew more vertical. There was less tendency for stems on the periphery of the clump to bend away from the other stems. This resulted in the shading and dying of some slower growing branches (See Plate V).

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[1] Whenever wilting was noted, the garden was watered by sprinkling. Wilting was first obvious in the plants treated with 100 ppm GA, and if the garden was not sprinkled within a short time, wilting would be seen in the plants treated with 10 ppm GA. No wilting was observed in the control plants at this stage.

PLATE V Growth habit of peppermint plants one month following treatment. The plants from left to right received 100, 10, and 0 ppm GA.

INVESTIGATION II. EFFECT OF GIBBERELLIC ACID ON HISTOLOGY OF PLANTS

A. EFFECTS ON LEAVES

Histological sections of peppermint leaves were examined by Youngken (1948) and the following description given:

1. UPPER EPIDERMIS composed of large, clear epidermal cells with wavy vertical walls and possessing few or no stomata.

2. PALISADE PARENCHYMA, comprising a layer of columnar cells rich in chloroplasts.

3. SPONGY PARENCHYMA, of about 5 layers of irregular shaped chloroplastid-containing cells and intercellular-air-spaces. Through this region the veins course.

4. LOWER EPIDERMIS, of small epidermal cells with wavy vertical walls and numerous elliptical stomata. This epidermis in the region of veins and midrib exhibits as outgrowths non-glandular and glandular hairs. The non-glandular hairs are uniseriate, papillose, 1- to 8-celled. The glandular hairs have a 1- to 2-celled stalk and a 1- to 8-celled glandular head. These hairs contain the oil of peppermint. In the drug obtained from cultivated plants, the terminal cell of the non-glandular hair often becomes glandular.

The glandular trichomes seen were of two distinct types, one with a 1-celled stalk and a 1-celled head and the other with a 1- to 2-celled stalk and a multicellular glandular head composed of 5 to 8 cells. The multicellular glandular trichomes were sunken so that only the large glandular top cell projected above the surface of the leaf. The trichomes were numerous near large veins, and were found on the upper as well as the lower epidermis.

Microscope slides of leaf cross sections were made from carefully matched leaves. The leaves had attained their maximum size, were from the middle nodes of the stems and were as similar as possible in every way save for the effects of the treatment given the plants.

All of the features described by Youngken were observed in every leaf examined; however, differences were noted between leaves of the plants receiving different treatments. Compared with the controls, the leaves from plants treated with 100 ppm GA were approximately one half as thick. The epidermal cells were slightly smaller; the palisade parenchyma cells were more nearly isodiametric rather than elongated; and the spongy parenchyma was composed of fewer and smaller cells. The stomates appeared equal in number and similar in shape, and the non-glandular trichomes were similar in number and shape. The small, two-celled glandular trichomes appeared to be not as well developed and lacked the swollen appearance of the glandular head cell. The glandular trichomes with multicellular heads were similar in appearance and number. Plate VI illustrates the differences observed between the leaves of plants treated with 0 and 100 ppm of GA. The leaves from plants treated with 10 ppm were intermediate in appearance between the two, but were more nearly like the 100 ppm GA-treated leaves.

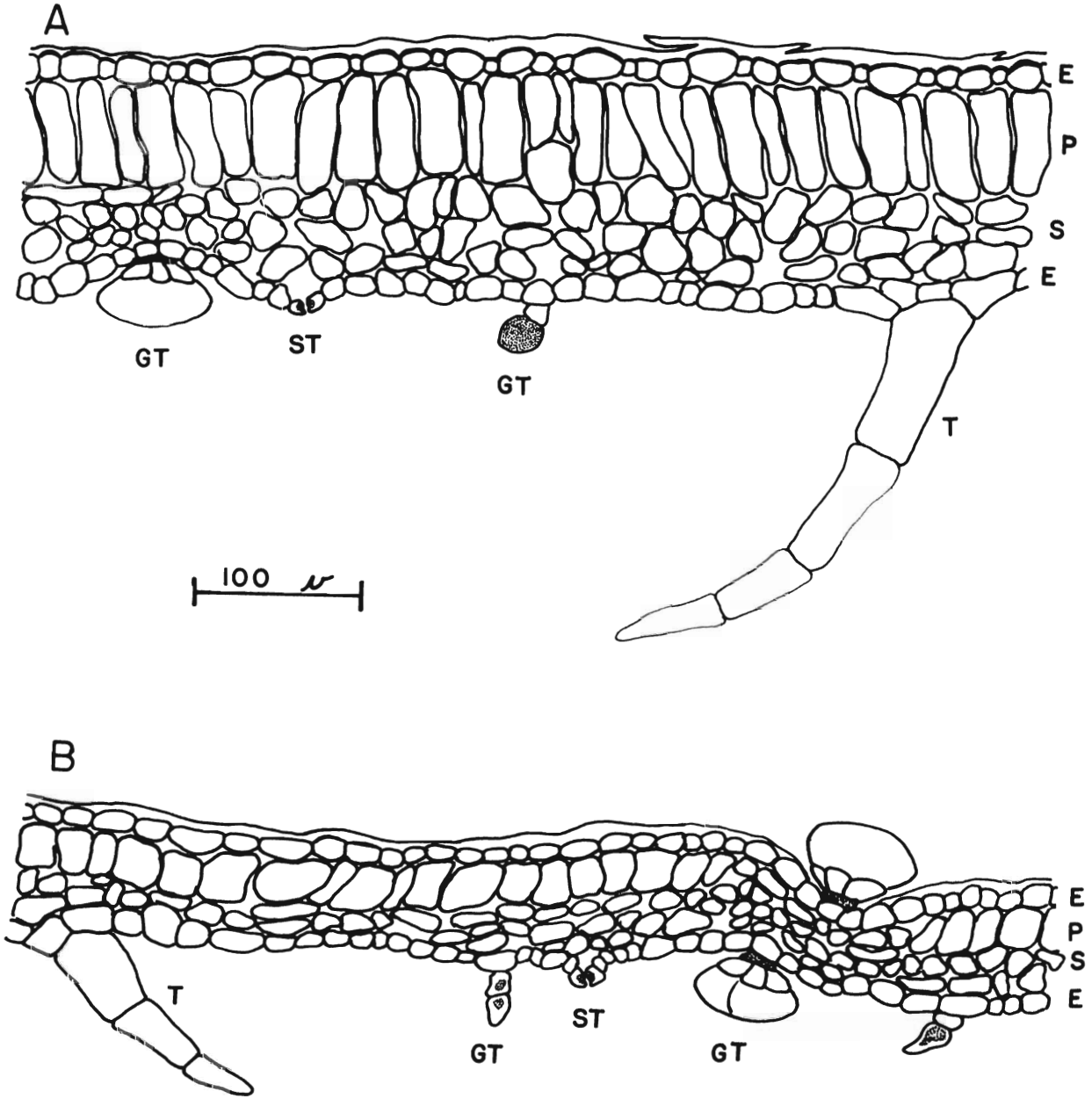


PLATE VI Leaf cross sections from (A) control plants and (B) from plants treated with 100 ppm GA. E = epidermis (The upper epidermis is at the top of the illustration); P = palisade parenchyma; S = spongy parenchyma; ST = stomata; T = trychome; GT = glandular trychome.

B. EFFECTS ON STEMS

Microscope slides of stem sections were made. The sections examined were made from the first flowering stalks to bloom and were from the middle internodes of the stems.

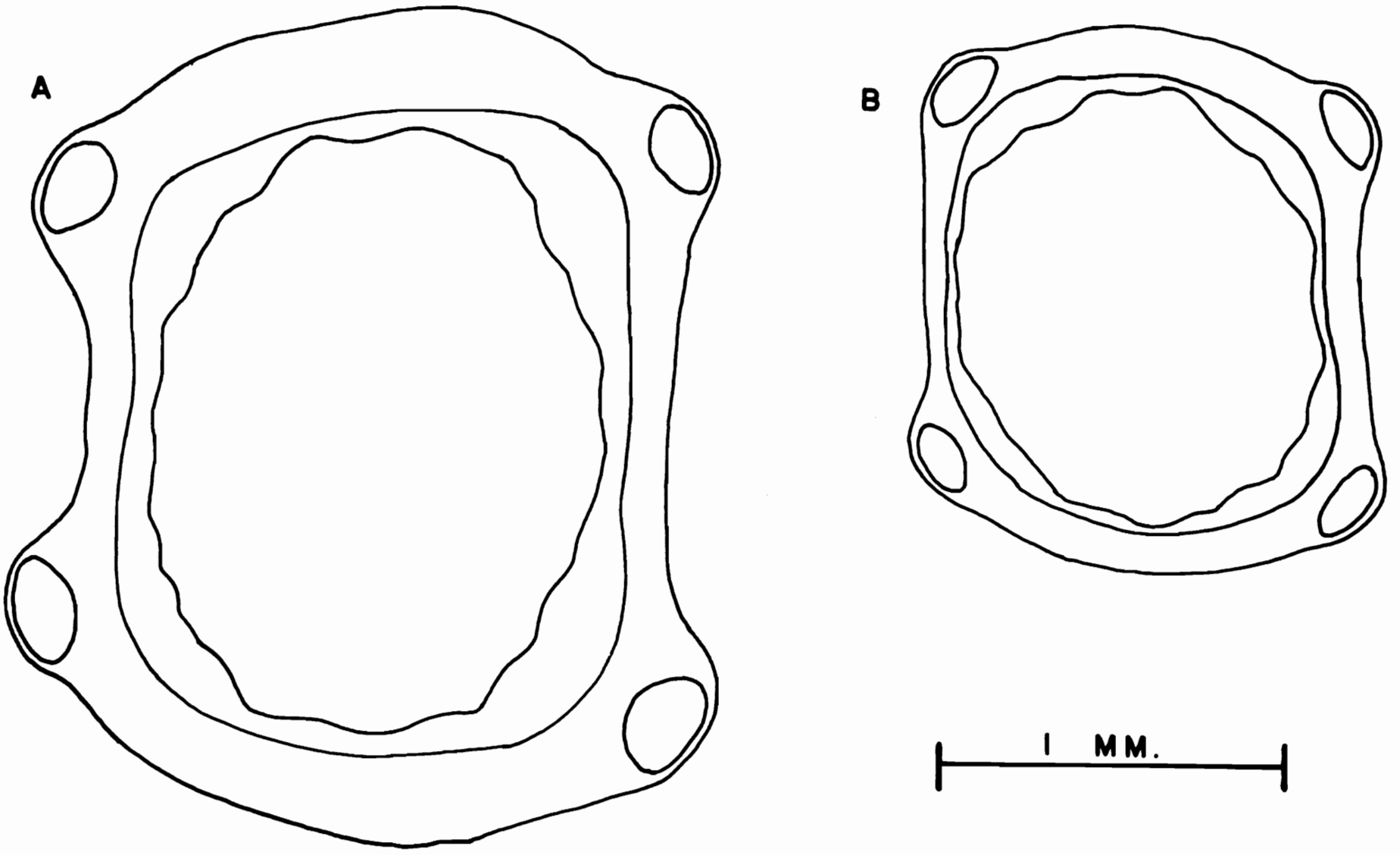
Stem cross sections from the control plants were quadrangular in outline with longitudinal bundles of colenchyma cells in each of the four corners. The epidermis was composed of a single layer of cells and contained slightly raised stomata. Three types of trichomes were seen: non-glandular uniseriate trichomes of from 1 to 8 cells; 1- to 2-celled glandular trichomes; and numerous glandular trichomes composed of a 1- to 2-celled stalk and a 2- to 8-celled head. The latter trichomes were only slightly sunken.

The cortex was composed of parenchyma from 8 to 12 cells thick, the outer cells containing chloroplasts. Air spaces in the cortex were about the same diameter as the cortical cells. A distinct endodermis was not recognized, but a one-celled thick layer of pericyclic tissue was seen. The largest vascular bundles were in the corners inside the colenchyma, but cambial growth had also started at a position intermediate between the corners, and, in some stems, cambial growth extended all around the stem. The phloem was composed of sieve tubes and companion cells; the cambial layer was not distinct, and the xylem was composed mainly of vessels and fibers. The pith was composed of isodiametric parenchyma cells and small air spaces. The cells at the periphery were slightly smaller than those in the central portion. (See Plate VII a and VII b)

Longitudinal sections of the stems of the control plants indicated in addition that the stomates were arranged in longitudinal rows. The air spaces in the cortex were elongated longitudinally. Vessels were mainly of the spiral type with a few anular vessels.

Compared with the stems of the control, sections of stems from plants treated with 100 ppm of GA showed the following changes:

(1) The stems were about 60 per cent as thick.
(2) The cells were smaller in size.
(3) There were fewer cells in all areas of the stem.
(4) The air spaces in the cortex were larger and were often 10 to 20 times as large as the surrounding cortical parenchyma cells.
(5) There was less secondary xylem and phloem.
(6) Some of the pith parenchymas cells had split from other cells leaving a hollow stem. This was more obvious in older stems.


A
B
1 MM.

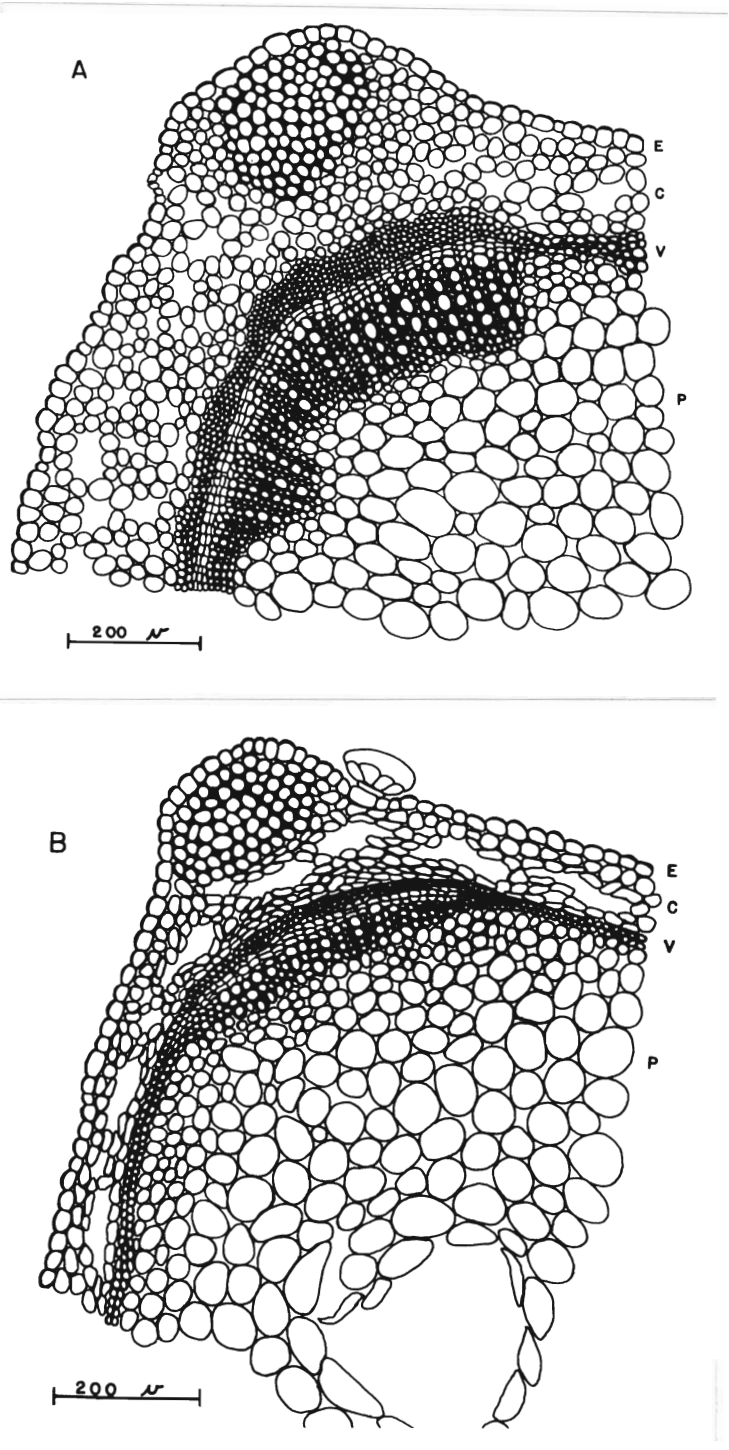


PLATE VIIb Stem cross sections from (A) control plants and (B) from plants treated with 100 ppm GA. A proportionately larger area of the latter is included to show the air space in the pith. E = epidermis; C = cortex; V = vascular tissue; P = pith.

(7) Longitudinal sections showed that the differences in cell shape were mainly those of cell elongation. Figure 9 shows the per cent increase in length over the control of the epidermal cells, cortical cells, and pith cells. It also shows the per cent increase in internode length. The greater stem elongation is therefore mainly, if not exclusively, due to cell elongation rather than to an increased number of cells.

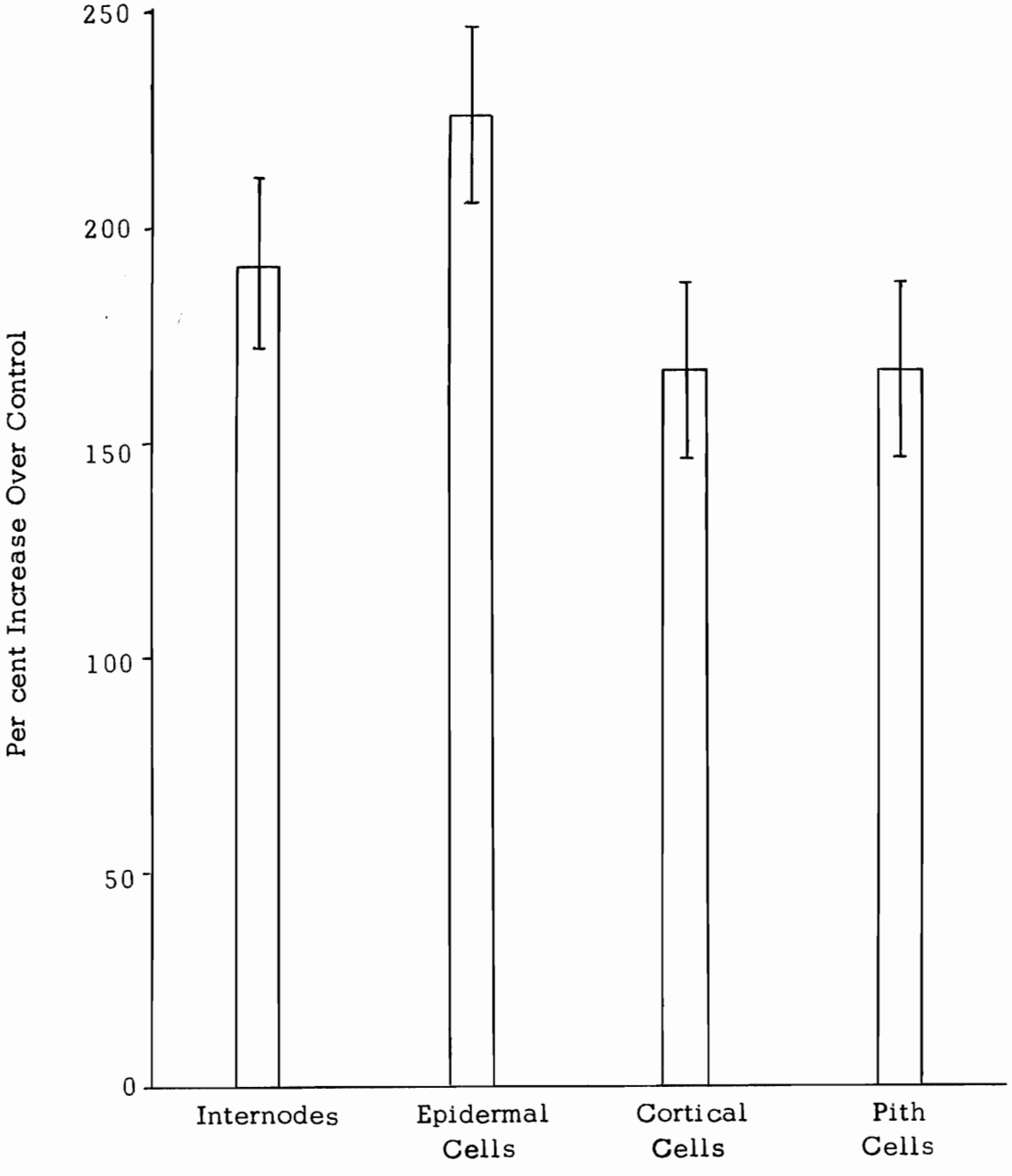


Fig. 9 Per cent increase over the control in length of internodes, epidermal cells, cortical cells, and pith cells of stems from plants treated with 100 ppm GA. See text for discussion.

C. EFFECT ON POWDERED HERB.

POWDERED PEPPERMINT. Powdered Peppermint is green to light olive-green. It shows fragments of leaf epidermis with wavy vertical walls and, if from the lower surface of the leaf, with numerous stomata and glandular and non-glandular hairs, the latter especially numerous along the veins; glandular hairs with a 1- to 2-celled stalk and 1- to 8-celled head, usually set in a depression in the leaf and containing volatile oil and frequently yellowish or brownish crystals which are birefringent; non-glandular hairs with thin, papillose walls and frequently with short, longitudinal striations of 1 to 8 cells and up to 1400μ in length, the terminal cell pointed or sometimes globular; fragments of chlorenchyma with vascular tissue, the vessels spiral or with simple pits and but slightly lignified; fragments of collenchyma and of thin-walled, non-lignified fibers associated with parenchyma. The pollen grains are spheroidal and smooth.[1]

Powdered peppermint grown in Utah, with or without GA treatment, corresponded to the U.S.P. XV description. Nothing was seen that could be used to distinguish the powdered GA-treated peppermint from the non-treated. Plate VIII shows the similarities between the various powdered samples.

[1]U.S.P. XV, 1955.

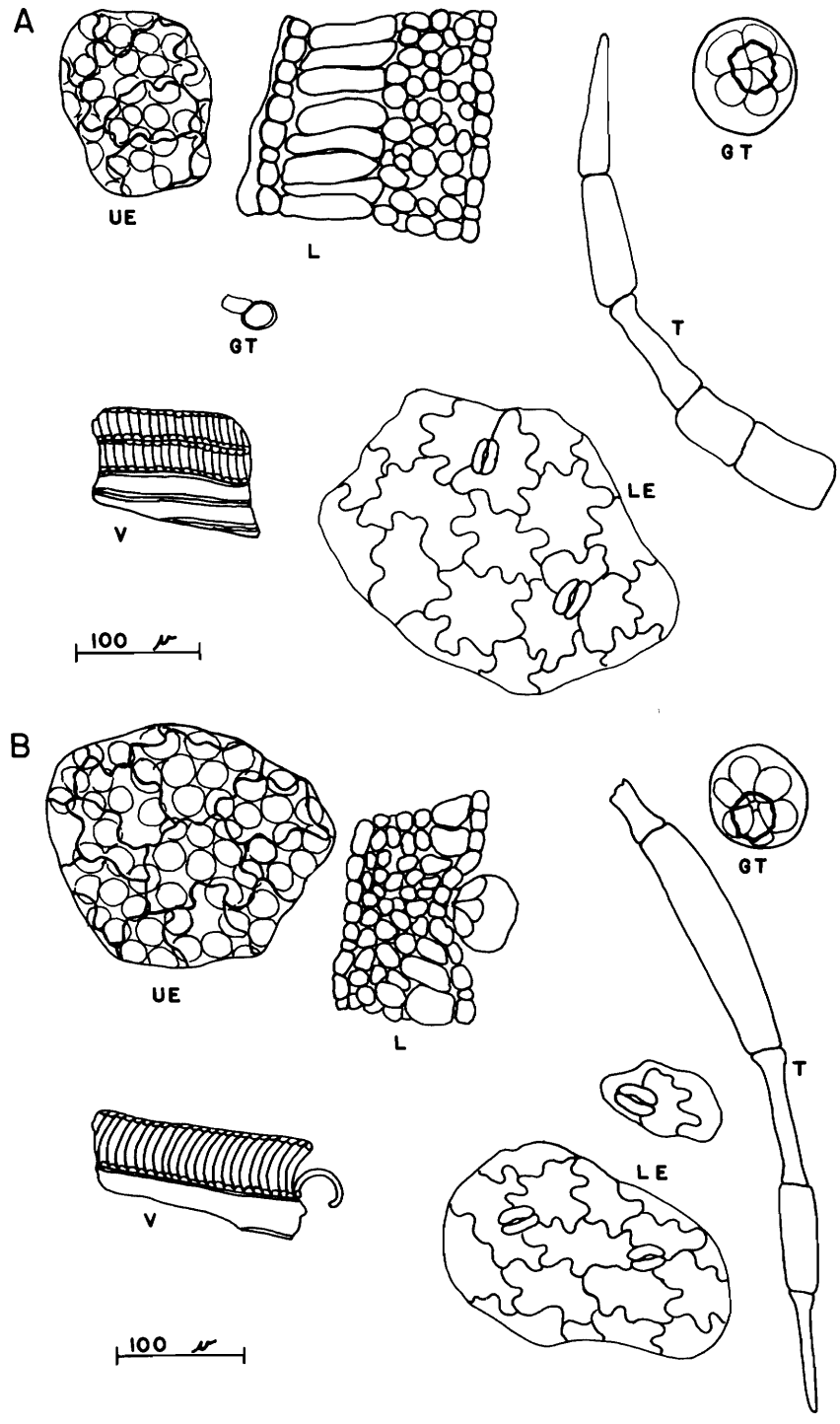


PLATE VIII Samples of powdered peppermint herb from (A) control plants and (B) from plants treated with 100 ppm GA. UE = upper epidermis of the leaf with palisade cells showing; L = leaf fragment; LE = lower epidermis of leaf showing stomata; T = trichome; GT = glandular trichome; V = vascular tissue.

INVESTIGATION III. EFFECT OF GIBBERELLIC ACID ON VOLATILE OIL

During the summer of 1958 and 1959 three collections of peppermint were made for the purpose of obtaining volatile oil for analysis. The volatile oil was distilled in a Loyd extractor the same day the peppermint was harvested. The first collection of oil was made July 29, 1958, and will be referred to as the "summer 1958 collection." The two other collections were made September 24, 1958, and June 24, 1959, and will be called the "fall 1958 collection" and "summer 1959 collection," respectively.

Following the rectification of the volatile oil samples by steam distillation, they were assayed for the chemical and physical properties indicated in the U.S.P. XV (1955).

PEPPERMINT OIL

It yields not less than 5 per cent of esters, calculated as menthyl acetate ($C_{10}H_{19}.C_2H_3O_2$), and not less than 50 per cent of total menthol ($C_{10}H_{19}OH$), free and as esters.

DESCRIPTION. Peppermint oil is a colorless or pale yellow liquid, having a strong, penetrating odor of peppermint, and a pungent taste, followed by a sensation of cold when air is drawn into the mouth.

SOLUBILITY IN ALCOHOL. One volume of peppermint oil dissolves in 3 volumes of 70 per cent alcohol.

SPECIFIC GRAVITY. The specific gravity of peppermint oil is not less than 0.896 and not more than 0.908.

OPTICAL ROTATION (page 807). The optical rotation of peppermint oil is not less than -18° and not more than -32° in a 100-mm. tube.

REFRACTIVE INDEX (page 935). The refractive index of peppermint oil is not less than 1.4590 and not more than 1.4650 at 20°.

SPECIFIC GRAVITY

The specific gravity of each of the oil samples was determined in a specific gravity bottle at 20°C. A 10 ml. Gay-Lussac specific gravity bottle was used for most of the oils. For the oils where less than 10 ml. of oil was available, a smaller bottle was used. Weights of the empty bottle, bottle filled with the oil being tested, bottle filled with H_2O, and bottle filled with Mercury were used to calculate the specific gravity. All weighings were carried out on an analytical balance and were calculated to the fourth decimal. The bottles were dried to constant weight between weighings.

In Table 14 the values for specific gravity may be seen. In each case the oil from the GA-treated peppermint plants had a higher specific gravity than the oil from the control plants. Many of the peppermint oils produced in Utah were not within the limits set by the U.S.P.

Table 14

Specific Gravity

Treatment	Specific Gravity
Summer 1958 collection	
0 ppm	0.9194*
10 ppm	0.9273*
100 ppm	0.9303*
Fall 1958 collection	
0 ppm	0.9136*
10 ppm	0.9545*
100 ppm	0.9277*
Summer 1959 collection	
0 ppm	0.8982
10 ppm	0.9030
100 ppm	0.9095*

*Not within U.S.P. specifications.

OPTICAL ROTATION

The optical rotation for each oil sample was determined at 25°C. in a Rudolph polarimeter of the half-shadow type. A tube 100 mm long and about 9.5 mm inside diameter was used in most determinations. Due to the small quantity of oil available, the determination of optical rotation of two oils was made in a tube 100 mm long and about 1.5 mm inside diameter.

It may be seen from Table 15 that the oil from plants treated with GA were generally more levo rotatory than the oil from control plants. None of the oils from the fall 1958 collection were within U.S.P. specifications.

Table 15

Optical Rotation

Treatment	Optical Rotation
Summer 1958 collection	
0 ppm	- 31.3°
10 ppm	- 30.5°
100 ppm	- 25.3°
Fall 1958 collection	
0 ppm	- 40.3°*
10 ppm	- 35.9°*
100 ppm	- 40.7°*
Summer 1959 collection	
0 ppm	- 32.2°*
10 ppm	- 29.1°
100 ppm	- 28.2°

*Not within U.S.P. Specifications.

REFRACTIVE INDEX

The refractive index of the oil samples was determined by the use of an Abbe Refractometer at 20°C. From Table 16 it may be seen that the refractive index for oils from GA-treated plants is higher than oil from the control plants.

Table 16

Refractive Index

Treatment	Refractive Index
Summer 1958 collection	
0 ppm	1.4616
10 ppm	1.4631
100 ppm	1.4661*
Fall 1958 collection	
0 ppm	1.4598
10 ppm	1.4680*
100 ppm	1.4600
Summer 1959 collection	
0 ppm	1.4605
10 ppm	1.4640
100 ppm	1.4640

*Not within U.S.P. specifications.

PER CENT ESTERS

In the assay of the oil samples for per cent esters, the U.S.P. method was followed with the exception that about 5 Gm. of oil was used rather than 10 Gm. samples. From Table 17 it may be seen that the oil from plants treated with 100 ppm of GA had the highest per cent of esters and that in each case the oil from the control plants has the lowest.

Table 17

Treatment	Per Cent Esters
Summer 1958 Collection	
0 ppm	9.78
10 ppm	14.86
100 ppm	19.45
Fall 1958 Collection	
0 ppm	12.36
10 ppm	20.87
100 ppm	31.35
Summer 1959 Collection	
0 ppm	10.86
10 ppm	12.09
100 ppm	13.16

PER CENT OF TOTAL MENTHOL

Because of the small samples available for the total menthol determination, an assay by Mason (no date) was used which required smaller quantities of oil than the U.S.P. assay. The percentage of free alcohol was first determined as follows:

DETERMINATION OF FREE ALCOHOL

Weigh accurately in a small vial approximately 1.000 Gm. of the sample, place the vial and oil in an acetylation flask, add exactly 5.00 ml., accurately measured from a burette, of a freshly prepared acetylating mixture consisting of four parts by volume of n-butyl ether and one part of acetic anhydride and mix the contents of the flask thoroughly in order that the oil is in complete contact with the acetylating mixture. Prepare a blank in an identical manner, omitting the oil. Connect the air condenser and boil the contents of the flask gently for 1 hour on a sand bath. Add 20 ml. of hot water through the condenser, boil vigorously for an additional 1/2 hour to hydrolyze the excess acetic anhydride, remove the flask, allow to cool, and add 20 ml. of cold water through the condenser. Remove the flask from the condenser, rinse the ground glass joint with a wash bottle, allowing the washings to run into the flask, add 8-10 drops of phenolphthalein T.S., and titrate the excess acid to the full red color of the indicator with N/2 alcoholic KOH, titrating the blank first and matching the color in the oil sample with the blank.

Calculate as follows:

$$\frac{\left(\frac{\text{Mol. wt. of alcohol}}{20}\right) \text{X ml. of N/2 KOH}}{\text{Wt. of sample}} = \% \text{ Free alcohol}$$

The percentages of free alcohols and of total esters were added to get the per cent total menthol. Table 18 shows that the amount of free alcohol in the oils from GA-treated plants was generally higher than from the control plants. The total menthol was higher in all samples.

Table 18

Per Cent Free Alcohol and Per Cent Total Menthol

Treatment	Free Alcohol	Total Menthol
Fall 1958 Collection		
0 ppm	58.8	71.2
10 ppm	67.0	87.9
100 ppm	46.2	77.6
Summer 1959 Collection		
0 ppm	44.5	55.4
10 ppm	45.8	57.9
100 ppm	52.2	65.4

(The oils from the Summer 1958 collection were lost in the determination, and additional oil was not available.)

ACIDITY

During the assay for total esters the author noted that larger quantities of base were required to neutralize the excess acid in the oils from GA-treated plants. To confirm this observation, 2 ml. samples of each oil were mixed with 10 ml. of neutralized alcohol. The amount of 0.1 N NaOH required to neutralize the acid was recorded using phenolphthalein T.S. as an indicator. From Table 19 it may be seen that the oil from GA-treated plants was more acid than the oil from control plants. Because the nature of the acid was not known, the amount of acid in the samples could not be calculated.

Table 19

Acidity

Treatment	Ml. 0.1 N NaOH Required to Neutralize the Free Acid
Summer 1958 Collection	
0 ppm	0.2
10 ppm	0.8
100 ppm	4.5
Fall 1958 Collection	
0 ppm	0.5
10 ppm	3.5
100 ppm	5.2
Summer 1959 Collection	
0 ppm	0.2
10 ppm	0.3
100 ppm	0.4

OTHER PROPERTIES

All peppermint oil samples when freshly rectified were colorless, but small samples stored in clear glass containers in the laboratory for six months turned pale yellow. All of the oils had "a strong, penetrating odor of peppermint and a pungent taste, followed by a sensation of cold when air is drawn into the mouth" (U.S.P. 1955). There were, however, slight differences in the odor and taste of the various samples. In general the oils from plants treated with GA had less odor of peppermint, were less sweet smelling, and aromatic waters prepared from the oils were more bitter.

All of the oil samples corresponded to the solubility in 70 per cent alcohol as described in the U.S.P.

DISCUSSION

This investigation has shown that GA exerts a physiological effect on peppermint plants which results in a change in the growth habits of the plant and in the quantity and quality of the volatile oil produced. The increase in height, which was the most readily observed change, was due mainly to an increase in internode length. The small increase in the number of nodes observed was statistically insignificant. Based on the length of three types of cells: pith, cortex and epidermis, it would appear that the elongational effect of the stems was basically due to cell elongation rather than to an increased number of cells. A decreased number of cells and a reduction in the size of cells in the stem cross section resulted in a thinner and more spindly stem.

The lower leaves of the treated peppermint plant were larger in area but thinner in cross section; however, the upper leaves were smaller in area and thinner in cross section. The thinner leaves were more susceptible to wilting. The decreased number of vascular elements may have resulted in a reduced water conduction which could have contributed also to wilting.

Although the first, short term experiments (3 to 5 weeks) indicated that an increase in the fresh and dry weight of peppermint herb could be anticipated this was not borne out in long term field experiments. The initial increase in vegetative growth that resulted from the first application of GA appeared to be followed by a subsequent decrease in growth and vigor. An even greater decrease in growth resulted when the tops were harvested and the plants allowed to recover. Experiments by other authors have yielded similar results, for example, experiments with hay (Stowe and Yamaki,1959) have led to the conclusion that the decrease obtained in the second cutting of hay offsets the value of the increase in the first cutting following application of GA to that crop. Apparently the same effects hold true for peppermint.

The latent decrease in growth may result from the failure of the roots to obtain sufficient mineral nutrients or water to sustain the rapidly growing tops. At the present time there is a discrepancy as to whether or not root growth is actually decreased by the use of foliar sprays of GA or whether the normally-growing roots fail to keep pace with the tops (Brian,1958). Whichever is the case, a number of reports have indicated that GA-treated plants frequently respond with an increase in vegetative growth when fertilizers are applied (Wittwer and Bukovac,1958).

Detailed studies are lacking regarding the proper balance between GA application and fertilizer treatment required to obtain the maximum growth increase of crops under agricultural conditions.

Haber and Tolbert (1957) came to the conclusion that "Gibberellic acid did not enhance the rate of CO_2 fixation per unit of leaf tissue and did not alter the general pathways of short-term metabolism of the newly fixed $C^{14}O_2$ in the sugars, organic acids and amino acid products." The growth habit of the peppermint plants, however, may have limited total photosynthate production since the stems of GA-treated plants grew almost straight up. This resulted in the shading of many lower leaves, and some of the shorter branches toward the center of the clump ceased growing after the first few weeks. Untreated peppermint plants "spread out" so that there were fewer branches and leaves in the shade of other plant parts (See plate V).

The decrease in rhizome production in the GA-treated plants could partially account for the decrease in vigor observed following the harvest of the leaves and flowering tops. The aerial stems may have elongated abnormally at the expense of other growing areas such as the rhizomes. Increased apical dominance resulting in a reduction of branching has been observed in a number of plants (Brian, 1959).

Taking into account the possible effects of a decreased root/shoot ratio, the effects of shading, and the effects of apical dominance, the author feels that the reduction in weight is not fully accounted for. A more adequate explanation can be found in the theories regarding the mechanism of action of GA. Brian (1959) has proposed a theory regarding the interrelationship between GA and the auxin, indolacetic acid (hereafter abbreviated IAA). In brief, it has been proposed that the growth response of GA is mediated through IAA, that the growth-stimulating action of IAA is limited by an inhibitor (which as yet has not been identified[1]) and that a GA-like substance, either natural or applied, acts to inhibit the IAA inhibitor.

Weijer (1959) in work with GA and IAA on Impatiens (Impatiens balsamina) found that GA plus relatively small amounts of IAA produced elongation greater than that produced by GA alone, but with increasing concentrations of IAA the response to GA was decreased. IAA alone produced slight stunting in this instance. In regard to stem elongation, Weijer concluded that "At relative high auxin levels the production of auxin inhibitor is so greatly

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[1] Pilot (1957) has suggested that IAA oxidase is the inhibitor, but Brian (1959) has given evidence which indicates that some other inhibitor must also be involved.

accelerated that there is more auxin inhibitor present in the plant tissue than there is auxin" and "It seems that application of additional auxin produces a rapid build-up of the auxin inhibitor(s) in the plant."

Despite the paucity of experimental evidence to substantiate the theory proposed by Brian, the theory does not conflict with the results obtained by the author. There was an indication in these experiments that on prolonged use of GA there was an increase in IAA inhibitor, which would agree with this concept.

In the experiments to determine the length of time that one dose of GA exerted its effect, it was noted that for a few weeks following treatment, the internodes produced were longer than the internodes of the control plants, but later the internodes produced were shorter than the control. In the case of the 10 ppm GA treatment, four or five internodes were longer than normal. This was followed by a decrease in extent of elongation which, in some stems resulted in a loss of apical dominance and a cessation of apical growth. In the 100 ppm GA treatment, seven to nine nodes were of an increased length. This was likewise followed by a period of time during which shorter than normal internodes were produced. The reduction in length of internodes being produced appeared no more severe in the plants treated with 100 ppm of GA than in plants treated with 10 ppm. It would appear, therefore, that (1) the effect of the IAA inhibitor extends for a time after the effect of GA has ceased, and (2) the increase in IAA inhibitor is not directly proportional to the amount of GA used. Further experimentation would be necessary to confirm these observations.

As previously noted, GA-treated peppermint plants yielded a smaller quantity of oil than the control plants. Also, the percentage of certain constituents of the oil was altered. The explanation for this would be extremely difficult to determine since the biosynthesis of volatile oils and their function in plants is but poorly understood (Haagen-Smit, 1949).

The tests for physicochemical properties of peppermint oil as specified by the U.S.P. were run. Table 20 summarizes the difference. Only one of the oil samples was of U.S.P. quality and that one was obtained in the summer 1959 collection from plants treated with 10 ppm of GA. In addition to the changes listed, it was observed that the oil from GA-treated plants was more acidic. Since free acetic acid has been identified in peppermint oil samples (Guenther, 1949), it is possible that the increased acidity is due to acetic acid. This would help to account for the greater amount of esters calculated as menthyl acetate.

Reitsema (1958) has concluded that menthol is a derivative of Menthone and Rutovskii and Travin (1929) have reported that the menthone content decreases in peppermint oil as the menthol content increases. Further

Physical and Chemical Properties of Volatile Oil from Peppermint Plants

Property Treatment	Summer 1958 Collection	Fall 1958 Collection	Summer 1959 Collection
Specific Gravity			
0 ppm	0.9194*	0.9136*	0.8982
10 ppm	0.9273*	0.9545*	0.9030
100 ppm	0.9393*	0.9277*	0.9095*
Optical Rotation			
0 ppm	-31.3°	-40.3°*	-32.2°*
10 ppm	-30.5°	-35.9°*	-29.1°
100 ppm	-25.3°	-40.7°*	-28.2°
Refractive Index			
0 ppm	1.4616	1.4598	1.4605
10 ppm	1.4631	1.4680*	1.4640
100 ppm	1.4661	1.4600	1.4640
Per cent Esters			
0 ppm	9.78%	12.36%	10.86%
10 ppm	14.86%	20.87%	12.09%
100 ppm	19.45%	31.35%	13.16%
Total Menthol			
0 ppm	**	71.2%	55.4%
10 ppm	**	87.9%	57.9%
100 ppm	**	77.6%	65.4%

* Not within U.S.P. specifications.
** Oil lost during assay.

chemical analysis would be necessary to determine whether the amount of menthone-menthol was increased or whether there was a greater conversion of menthone to menthol. Many other biochemical problems beyond the scope of this research problem would probably yield information regarding the biosynthesis of various constituents of peppermint oil. Such studies, however, should be undertaken.

A decrease in peppermint herb and peppermint oil was observed following the application of GA to peppermint plants. At the present time no recommendation can be made for the application of this growth regulator in the commercial production of peppermint products.

Future research may reveal economically-important considerations other than herb and oil yield. For example, Guenther (1949) indicated that peppermint growing in rows "row mint" can be cultivated mechanically and can be weeded much easier than peppermint that has spread to produce a solid turf or mat "meadow mint." Because of the greater freedom from weeds, the "row mint" produces a superior product. Applications of GA might retard the spreading habit of mint so that cultivation and the less difficult weeding of peppermint could be accomplished for an additional year.

Guenther (1949) also stated that while about 20 chemical constituents have been isolated from peppermint "...comparatively little is known about those compounds which, although occurring in the oil in small quantities only (trace substances), are, nevertheless, chiefly responsible for its sweet odor and flavor, and distinguish it from the Japanese or Chinese mint oil (Mentha arvensis). The latter, despite its high content of menthol, possesses a somewhat bitter odor and flavor, noticeable particularly in the higher boiling fractions." Studies regarding the organoleptic properties might indicate that the alteration in chemical constituents could result in a superior product for some special use. It is not likely that the increase in menthol content will be economically important since certain varieties of Japanese mint (Mentha arvensis) have been reported to contain from 80 to 90 per cent menthol (Pratt and Youngken, 1956). Most of the "natural" menthol of the world comes from the Japanese mint (Claus, 1956).

It should not be surprising that few of the peppermint plants grown in Utah yielded oils that meet U.S.P. specifications. According to Turnow and Fischer (1948) peppermint oil obtained from plants grown in the eastern part of Washington, a dry region with a climate not greatly dissimilar from that of Utah, having an average rainfall of less than 6 inches per year, will not meet the U.S.P. requirements. According to Guenther (1949), many American oils contain less than 50 per cent menthol and therefore would not meet U.S.P. specifications. The varieties of peppermint grown in

various areas of commercial production have been selected and bred through many years in order to get the best suited strain for the particular area. None of the areas of commercial peppermint production have a climate similar to the climate around Salt Lake City. It would therefore be expected that in order to grow peppermint commercially in Utah a program of plant breeding would have to be undertaken to develop strains of peppermint suited to the area.

SUMMARY AND CONCLUSIONS

1. The morphological changes observed were characterized primarily by stem elongation and to a lesser degree by changes in leaf shape. The latter changes were related to the position of the leaf on the stem; the lower leaves were similar in shape but larger in surface area than leaves of control plants, whereas the higher leaves were more lanceolate and smaller in area.

2. The fresh weight yield of peppermint herb was reduced significantly but the dry weight yield of peppermint was not significantly different from that of the control plants.

3. Following harvest of the herb of treated peppermint plants, a new crop of peppermint was allowed to grow without further treatment. The second crop from GA-treated peppermint plants was markedly smaller than the crop from the control plants.

4. The yield of oil was reduced when compared on both a fresh weight and dry weight basis.

5. A single spraying of GA produced stem and internode elongation which was followed by a reduction in the rate of stem elongation and in the length of the internodes being produced. In some stems this reduction in elongation was accompanied by loss of apical dominance.

6. From observations of histological sections of the stem it was concluded that cell elongation primarily accounts for the change in internode length. There was no significant increase in the number of cells in the longitudinal direction.

7. In the stem cross section, a decreased number of cells in all tissues was observed. The reduction of cells resulted in a thinner stem.

8. Leaf cross sections revealed that the leaves were thinner than the leaves of the control plants. Changes in the shape of the cells were observed.

9. The powdered herb obtained from plants treated with GA was indistinguishable from the powdered herb of control plants or from powdered U.S.P. peppermint.

10. Assays were run on the oils obtained from the treated peppermint plants for the physical and chemical properties specified by the U.S.P. under peppermint oil. In general, the oils from plants treated with GA had higher specific gravities than the oils from control plants, were more levo-rotatory, had higher refractive indicies, and contained larger percentages of esters calculated as menthyl acetate and of alcohol calculated as menthol. Only one oil produced was within U.S.P. specifications and that was from a plant sprayed with 10 ppm of GA and harvested in the summer of 1959.

11. From the observations made to date, no recommendation can be made for the use of GA on peppermint crops. Further studies should be run to evaluate the effects of other concentrations than those used and other methods of application. Because the control peppermint plants grown in Utah did not yield U.S.P. grade oil, any further work done should be in an area of commercial peppermint production. It would appear, however, that 10 and 100 ppm GA as weekly sprays is either too high a concentration or is too frequent a spraying schedule for optimum production of peppermint herb or peppermint oil.

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